Department of Molecular Biosciences
College of Liberal Arts and Sciences

Molecular Biosciences Symposium 2023

August 18, 2023
Burge Union, Forum C/D
9:00 AM – 4:00 PM

This Symposium is Hosted by the 4th-Year Graduate Students and Sponsored By:

KU Chemical Biology of Infectious Disease
The University of Kansas
Center of Biomedical Research Excellence

The University of Kansas Cancer Center

K-INBRE
Kansas IDeA Network of Biomedical Research Excellence

MB GSO
Molecular Biosciences Graduate Student Organization

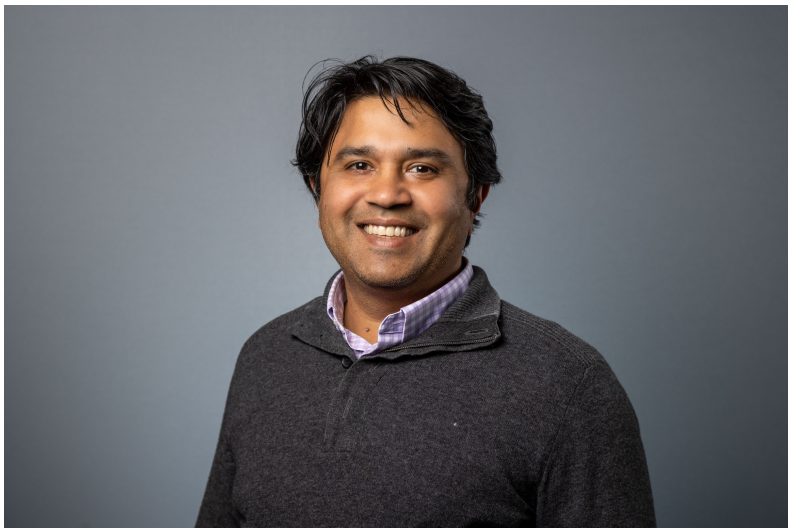
KU The University of Kansas
Center for Molecular Analysis of Disease Pathways

Symposium Schedule

Time	Event	Forum
8:30 AM	**Poster set-up** **Refreshments**	D C
9:00 AM	**Welcome** Introduction of new faculty & graduate students Acknowledgment of sponsors	C
	Faculty Talks	
9:10 AM	**Dr. Michael S. Wolfe** In Search of the Pathogenic Trigger of Alzheimer's Disease	C
9:40 AM	**Dr. Berl R. Oakley** Engineering fungi to up-cycle plastics	
10:10 AM	**Dr. Joanna S.G. Slusky** General features of outer membrane proteins	
10:40 AM	**Break**	
	Keynote Speaker	
10:50 AM	**Dr. Sagar D. Khare** Illuminating and designing specificity of protein interactions using computation and experiments	
11:50 AM	**Lunch**	C/D
	Student Talks	
1:00 PM	**Eldric Carreon** (Neufeld Lab) Determining Nuclear APC's role in UV-induced DNA damage response	C
1:20 PM	**Katherine Hanson** (Macdonald Lab) Extreme QTL Mapping Reveals Zinc Toxicity Resistance Loci	
1:40 PM	**Joseph O'Connor** (Fehr Lab) The Binding Affinity of Coronavirus Macrodomain Mac1 Counters an Innate Restriction of RNA Replication	
2:00 PM	**Jacob Kroh** (De Guzman Lab) Discovery of a Novel Membrane Interaction In The N-terminal Domain Of The Shigella Major Translocase IpaB.	
2:20 PM	**Break**	
	Poster Presentations	
2:30 – 3:15 PM	**Poster Session I** Odd number posters	D
3:15 – 4:00 PM	**Poster Session II** Even number posters	
5:30 PM	**Molecular Biosciences Social and Picnic** Holcolm Park	

Keynote Speaker

Dr. Sagar D. Khare



Rutgers University
Department of Chemistry and Chemical Biology

Illuminating and designing specificity of protein interactions using computation and experiments

Abstract:

Selective molecular recognition of cognate binding partners and substrates (and non-recognition of non-cognate interaction partners) by proteins is a key feature of all natural biological processes and is generally a result of evolutionary selection pressure. The ability to predict and design interaction specificity of proteins has wide-ranging applications for biotechnology and medicine. I will describe three recent efforts from our group in which we use a combination of structural modeling, machine learning and wet-lab experiments to illuminate the molecular basis of natural or engineered interaction specificities and use the developed models to obtain bespoke functional proteins. Implications for biocatalysis, therapeutics and molecular evolution will be discussed.

Poster Session Roster

No.	Presenter	Title
1	Parker Sperstad	Mechanistic Insights into the 2-step Dimerization Process of the Hepatitis C Virus RNA
2	Catherine Kerr	ADP-ribosylation-dependent immune responses restrict Mac1 mutant coronavirus in cell culture and tissue-specific manner
3	David Johnson	Computational Chemical Biology Core: A Chemical Biology of Infectious Disease COBRE Core Laboratory
4	Jennifer Hackett	Next Generation Sequencing at KU Genome Sequencing Core
5	Peter McDonald	Flow Cytometry Core: A Chemical Biology of Infectious Disease COBRE Core Laboratory.
6	Justin Douglas	The Nuclear Magnetic Resonance Core lab
7	Anu Roy	High Throughput Screening and Infectious Diseases Assay Development Laboratory at the University of Kansas
8	Ryan Grigsby	Equipment and Services of the KU Nanofabrication Facility
9	Jess Pfannenstiel	The Development of Coronavirus Macrodomain Inhibitors That Impair Virus Replication in Cell Culture
10	Indeewara Munasinghe	The Synthetic Chemical Biology Core (Scb): A Resource For Research In Chemical Biology
11	Bunu Lama	Identification of molecular targets of PICH remodeling activity for faithful chromosome segregation
12	Alec Bevis	The Role of PTPN22 and its Autoimmunity Associated Minor Allele during Virus Infection
13	Anam Shaikh	Autoimmunity-associated allele of PTPN22 changes Dendritic Cell function during virus infection
14	Saeideh Nasiri	Bioactive small molecules produced by the gut commensal Enterocloster citroniae modulate Vibrio cholerae behavior
15	Vedant Jain	Genetic Screens to identify genes acting downstream of Mab-5/Hox in the posterior migration of QL neuroblast in Caenorhabditis elegans
16	Qi Zhang	Inhibition of HuR/CD147/IL-6 axis enhances anti-PD1 immunotherapy response in cancer
17	Steve Seibold	The University of Kansas Protein Structure and X-ray Crystallography Laboratory
18	Daniel Montezano	Distribution and Features of Beta-Barrels in Bacteria
19	Asbin Chand	Extract of C. parqui reduces the proliferation of triple negative breast cancer cells MDA-MB-231 in vitro
20	Bikash Pokhrel	MUSASHI1 Affects Intestinal Epithelium Growth By Regulating MTORC1 Signaling
21	Emily Proctor	Computational Prediction of Chloroplast Outer Envelope β-barrel Proteins

Poster Session Roster

No.	Presenter	Title
22	Teja Nimmagadda	Computational Identification of Strands in Beta-Barrels using AlphaFold2 Predicted Protein Structures
23	Eldric Carreon	Determining APC's role in UV-induced DNA damage response
24	Lanjing Wei	Functional inhibition of the RNA-binding protein HuR sensitizes triple-negative breast cancer to chemotherapy
25	Kent Mulkey	Towards a DNA virus model system in Drosophila, Replication dynamics of a DNA virus in insect cell culture
26	Brielle McKee	Adaptive Mutations in LasR and FusA1 Modulate Ceftazidime Resistance of Pseudomonas aeruginosa
27	Diego Prieto	Determining the Importance of RsbV1's Phosphorylation State for Chlamydia trachomatis¬í Growth and Progeny Production
28	Jennifer Amrein	Development and Disease: Stress Response Effects on Synaptic Loss in C. elegans
29	Kervens Accilien	Differential Evolution of Pseudomonas against Ribosome-Targeting Antimicrobial Peptide vs Small Molecule Antibiotic
30	Andrew Daufel	Structures of TolC Bound to Bacteriocins
31	Maxim Rodzkin	Role of specific CDKs in HSV-1 lytic infection
32	Andrew Evans	Inhibition of XPO1 in Colorectal Cancer
33	Alex Bowman	OmpA Folding is Complex and Robust
34	Ryan Feehan	Using AlphaFold2 to predict RME-8 self-oligomerization
35	Maggie Schedl	Toward a dsDNA Virus Model System in Drosophila: Investigating the Dynamics of the Drosophila innubila Nudivirus Grown for One Year in Cell Culture
36	Anika James	The role of nuclear APC in regulating MUC2 expression and colonic inflammation
37	Abdulrahman Naeem	Host Glycan Utilization is Influenced by Multiple Transcription Factors in Enterococcus faecalis
38	Xiaoqing Sarah Wu	Preclinical Core Facilities
39	Rik Dhar	Engineering a GFP-Mimetic Outer Membrane Protein through Rational Design and Deep Learning
40	Evan Schulz	Targeting EWS-dependent pathways as chemotherapeutic vulnerabilities in Ewing sarcoma
41	Alicia S Brown	Quorum sensing control of aminoglycoside antibiotic resistance in Pseudomonas aeruginosa
42	Sutton Stegman	Ironing Out a Role for HAF-6 in C. elegans

Poster Session Roster

No.	Presenter	Title
43	Mariana Ochoa	Developing a Novel Assay for Transposon Activity and the Influence of Iron Trafficking
44	Nilanjan Roy	Horizontally transmitted avirulent Nora virus is associated with upregulation of canonical immune pathways in cells of the fat body.
45	Maximino Emerson	Investigation of In Vivo Dimerization and Subcellular Localization of HAF-6 in C. Elegans
46	Nathan Smith	Induction of pilus gene cluster involved in interspecies interactions by acyl-homoserine lactone-dependent eavesdropping in Chromobacterium subtsugae

A Special Thanks to Our Sponsors

KU CHEMICAL BIOLOGY OF INFECTIOUS DISEASE
The University of Kansas
Center of Biomedical Research Excellence

The goal of the CBID is to provide the necessary mentor support and infrastructure to ensure the success of junior investigators. and to create a center that encourages basic research scientists to discover Chemical Biology of Infectious Disease.

The University of Kansas Cancer Center is on an urgent journey to break the devastating grip of cancer. We are the only National Cancer Institute-designated comprehensive cancer center in the region, and 1 of only 53 in the nation, to receive this elite distinction. Comprehensive designation is the highest level of recognition awarded by the NCI. It is the gold standard of excellence, awarded only to cancer centers with the deepest and broadest knowledge of cancer.

THE UNIVERSITY OF KANSAS
CANCER CENTER

KU THE UNIVERSITY OF KANSAS
Center for Molecular Analysis of Disease Pathways

Enabling technologies are critical for the understanding of the biochemical and physical basis of disease as well as its diagnosis. The COBRE CMADP pursues health-related research that is focused on the development and application of these technologies to understanding disease processes. Two of the Center's major missions are to recruit, support and mentor outstanding junior faculty investigators, and to establish, operate, and grow successful Core Labs that provide state-of-the-art capabilities and services to investigators.

The purpose of the Kansas INBRE (K-INBRE) is to promote multidisciplinary research networks with a focus on Cell and Developmental Biology; increase the research base and capacity through research support; provide a range of basic science and clinical research opportunities for student trainees; serve as a pipeline for students to continue in health research careers in IDeA states; and enhance science and technology knowledge of the state's workforce.

K·INBRE
Kansas IDeA Network of Biomedical Research Excellence

MB GSO
Molecular Biosciences Graduate Student Organization

Our organization strives to enrich the lives of graduate students by providing a sense of community through social activities, opportunities to learn about a broad range of science careers and promoting opportunities for graduate students to share their love of science with members of the community.

Poster Abstracts

1. Mechanistic Insights into the 2-step Dimerization Process of the Hepatitis C Virus RNA Genome

Parker D. Sperstad, Erik D. Holmstrom

The 3'X RNA of the Hepatitis C Virus (HCV) is a highly conserved, 98-nt sequence located at the 3' terminus of the HCV genome. This region of the genome can engage in several viral RNA-RNA binding interactions that have previously been shown to be critical for regulating translation, replication, and viral particle assembly. Within the 3'X RNA there is a stretch of 16 nucleotides that is responsible for two different RNA-RNA binding interactions: one heterotypic and the other homotypic. Notably, the nucleotide sequence for the heterotypic interaction is fully nested within the homotypic interaction and therefore formation of one interaction precludes formation of the other. Although these two interactions have important regulatory roles, it is not known what determines which interaction is formed at any given time and how they might interconvert. Using single-molecule FRET (smFRET) and size-exclusion HPLC (SE-HPLC) techniques to monitor the conformational status of the 3'X RNA under a variety of conditions, we are beginning to resolve these unanswered questions. First, in the absence of either binding partner, we observe two distinct conformations of monomeric 3'X with their relative abundance dictated by either solution conditions (e.g., [Mg2+]) or nucleotide deletions. Additionally, we observe a large conformational change in the 3'X RNA when exposed to its homotypic binding partner, resulting in the formation of a homodimeric extended duplex of the 3'X RNA. These findings support an emerging hypothesis that the conformation changes associated with the 3'X RNA function as riboregulatory switches that halt protein and RNA synthesis, ultimately giving way for virus particles to begin to assemble.

2. ADP-ribosylation-dependent immune responses restrict Mac1 mutant coronavirus in cell culture and tissue-specific manner

Catherine M. Kerr, Srivatsan Parthasarathy, Nancy Schwarting, and Anthony R. Fehr

ADP-ribosylation is a post-translational modification catalyzed by ADP-ribosyltransferases (ARTs), also called PARPs. Many of the 17 mammalian PARPs are interferon-stimulated genes (ISGs), indicating a prominent role for PARPs in the anti-viral response. All CoVs encode for an enzyme, Mac1, that can both bind to and hydrolyze ADP-ribose from proteins, indicating that CoVs may be especially sensitive to PARP activity. Using an encephalitic strain of mouse hepatitis virus (MHV), we found that PARP activity restricted the replication of a Mac1 mutant MHV (N1347A) in bone marrow-derived macrophages (BMDMs). An siRNA screen initially indicated that PARP12 may be capable of restricting N1347A replication. To confirm and expand upon these results, we generated PARP12-/- mice. While there was little effect on the replication of the WT virus, N1347A MHV replication was enhanced to near WT levels in PARP12-/- BMDMs. We hypothesized that these mice might be more susceptible to infection with the N1347A MHV than the WT virus. However, following intranasal infection, N1347A replicated poorly in both PARP12+/+ and PARP12-/- mice. In addition, there was no significant difference in weight loss or survival between PARP12+/+ or PARP12-/- mice infected with N1347A, indicating that other PARPs likely are able to functionally compensate for the lack of PARP12 in the brain. Further, immunohistochemistry and qPCR of infected brains suggest that N1347A is highly restricted in olfactory bulbs, an important site of COVID-19 pathogenesis. This data suggests that an ADP-ribosylation-dependent immune response in the olfactory bulb represses Mac1 mutant MHV.

3. Computational Chemical Biology Core: A Chemical Biology of Infectious Disease COBRE Core Laboratory

David K. Johnson

Part of the Chemical Biology of Infectious Disease COBRE at the University of Kansas, the Computational Chemical Biology Core (CCB) works in collaboration with the Molecular Graphics and Modeling (MGM) Laboratory to provide the computational resources and expertise to enhance the productivity of researchers studying infectious diseases, in addition to other projects. The CCB has the tools and expertise to perform virtual screening, small molecule docking, cheminformatics analysis of high-throughput screening hits, binding site prediction, protein/peptide/antibody modeling and docking (including Alphafold modeling), protein design, and molecular dynamics simulations.
Recent highlights include the identification inhibitors of ACMS decarboxylase and DNAJA1 via virtual screening, using modeling to identify the functional activity of Legionella pneumphila effector protein SidI, using modelling to assess the structural impact of clinically relevant point mutations of TRIM32, modeling the interaction between the Type III secretion system basal body and sorting platform proteins SctK and SctD from Pseudomonas aeruginosa, and the optimization of an inhibitor of PTPRD. With the software and expertise to perform virtual screening, protein-small molecule docking, protein/peptide modeling/docking, and cheminformatic analysis, the CCB is a valuable resource to enhance the productivity of researchers studying infectious diseases, in addition to other projects. The CBID COBRE is funded by the NIH NIGMS grant 1P20GM113117.

4. Next Generation Sequencing at KU Genome Sequencing Core

Jennifer L. Hackett, Kristen M. Cloud-Richardson, Erik A. Lundquist, Susan M. Lunte

The Genome Sequencing Core (GSC) is one of three research service core labs in the NIH COBRE Center for Molecular Analysis of Disease Pathways (CMADP) at the University of Kansas (KU). The major mission of the GSC is to provide researchers with next-generation sequencing (NGS) technologies. NGS, carried out in a massively parallel fashion, has been revolutionizing bio-medical research and used in a growing list of applications. Projects supported by the GSC include de novo genome assembly, genome re-sequencing for identification of mutations and polymorphisms, transcriptome analysis (RNA-seq), and epigenomic and gene regulation studies such as ChIP-seq, Methyl-seq, and small RNA analysis. The GSC enhances the genomics infrastructure already at KU by providing a range of Illumina sequencing platforms including the NextSeq 2000 and NextSeq 550 (mid-sized genome re-sequencing or transcriptome projects) and the MiSeq (metagenomic or targeted amplicon sequencing projects) to researchers at KU-Lawrence and across the region. To capture the full power of NGS, we provide a range of project support, including project consultation, sample quality check, sequencing library construction, Illumina sequencing, and FASTQ generation and demultiplexing. For latest pricing, current sequencing queue, or other information, visit the Genome Sequencing Core's website: https://gsc.ku.edu/.

5. Flow Cytometry Core: A Chemical Biology of Infectious Disease COBRE Core Laboratory.

Peter R. McDonald, Robin C. Orozco, and P. Scott Hefty

The University of Kansas Flow Cytometry Core (FCC) provides access to flow cytometry and cell sorting instrumentation and expertise to researchers. Services and training are provided for flow cytometry: cell sorting and multi-parametric analysis of individual cells in solution, calculated from their fluorescent or light scattering characteristics. The FCC provides assistance in sample processing, data analysis, instrument training, software support, method and grant assistance, manuscript support, and consulting. The FCC is a 980 ft2 BSL-2 facility equipped with a BD FACSymphony™ S6 Cell Sorter, a BD FACSAria™ Fusion cell sorter, a Cytek™ Aurora Spectral Flow Cytometer, and other supplemental assay instrumentation. The Cytek™ Aurora full-spectrum flow cytometer provides users with both tube-based and 96-well plate based spectral cytometry, with 5 lasers to allow analysis of 30+ colors. The BD FACS instruments allow measurement and sorting of up to 6 resolved populations of cells simultaneously, based on up to 50 parameters of detection using 18 simultaneous fluorochromes. The facility manages a FlowJo™ site license for data analysis software. The FCC will equip CBID researchers with tools directly applicable to infectious disease research, such as identifying and characterizing infectious agents such as bacteria, quantification and sorting of cells infected with microbial pathogens, and assessing chemical probe efficacy against infectious agents. The FCC resources enable monitoring immune responses and activation status associated with infection, and measuring changes in cellular phenotypes (size, granularity, complexity, density, expression) in response to compound treatment. The FCC seeks to assist CBID collaborators in achieving their research goals.

6. The Nuclear Magnetic Resonance Core Lab

Justin T. Douglas, Laurie Harned, Sarah Neuenswander

Nuclear Magnetic Resonance (NMR) spectroscopy is an analytical technique that is used to explore the structure and dynamics of molecules from drug-like small molecules to macromolecular complexes. At the University of Kansas our eight magnetic resonance spectrometers are managed as part of the NMR core lab. These instruments range from open-access "walkup" instruments for routine acquisition of data for organic structure elucidation to specialized spectrometers for characterization of paramagnetic species, such as transition metal complexes. The KU NMR core lab also manages spectrometers for biomolecular NMR and capabilities for studying solid samples. This poster introduces the staff of the lab, provides more detail on our instruments and explains to potential users how to exploit these state-of-the-art resources to answer their research questions.

7. High Throughput Screening and Infectious Diseases Assay Development Laboratory at the University of Kansas

Anuradha Roy, Ph.D.

The University of Kansas High Throughput Screening/Infectious Disease Assay Development Laboratory (KU-HTS/IDAD) is a state-of-the-art fee-for service facility, dedicated to providing academia, not-for-profit institutions, biotech, and pharmaceutical industries with exceptional assay development and high-throughput screening services at economical rates. KU-HTS/IDAD lab is equipped with cutting-edge instrumentation for liquid handling, compound management and signal detection via a variety of multimodal specialized readers. Clients have the option of using the KU/IDAD library of 400,000 small molecule, diverse, drug-like compounds and/or client's own compound collection. The staff has experience in executing cell-based, biochemical, siRNA as well as high content screening campaigns against a plethora of target classes. KU-HTS/IDAD is innovative and flexible in providing superior service to the drug discovery research community, including primary and secondary assay development, small to large compound screening (single concentration, qHTS and combination screens), compound profiling and data mining. KU-HTSL further leverages the strengths of the KU Core facilities and the KU Medical Center's Institute for Advancing Medical Innovations to support novel lead discovery research. The hits identified from screening campaigns serve as chemical probes that are useful in mechanism of action studies. Some probes can also serve as starting points for hit to Lead optimization studies leading to drug discovery.

8. Equipment and Services of the KU Nanofabrication Facility

Ryan Grigsby and Susan M. Lunte

The Kansas University Nanofabrication Facility (KUNF) is a Core Lab supported by the KU Office of Research and the Center for Molecular Analysis of Disease Pathways COBRE. The KUNF primarily caters to researchers who are manufacturing micro- and nanofluidic devices for biomedical research, but has the equipment and resources to accommodate broad research applications with micro- and nanofabrication needs. The core lab consists of about 1,300 ft2 of ISO class 5, 1,700 ft2 of ISO class 6 and 1,250 ft2 of ISO class 7 cleanroom space, housing tools and materials for techniques including photolithography, nano-imprint lithography, plasma (dry) etching (ICP-RIE), wet etching, metal and dielectric material thin film deposition, scanning electron microscopy (VP-SEM), contact angle goniometry, ellipsometry, profilometry, wafer dicing, laser ablation and engraving, 3D printing, hot embossing, and COMSOL software for device modeling. In addition, the facility has numerous microscopes for general inspection, ovens and furnaces, ultrapure water, dedicated process fume hoods and filtered lighting for photolithography.

This facility is under the direction of Dr. Susan Lunte. Services and usage of the facility are available to researchers from all Kansas universities. Training is provided to new investigators and graduate students in the use of micro- and nanofabrication procedures and equipment. In addition, researchers from both non-Kansas academic and private industry institutions may contract with the facility for consultation and services. Hourly and per-use rates apply for facility access, equipment usage, and staff labor. Consultation is free.

9. The Development of Coronavirus Macrodomain Inhibitors That Impair Virus Replication in Cell Culture

Jessica J. Pfannenstiel, Daniel E. Cluff, Lavania M. Sherrill, Anuradha Roy, Yousef M.O. Alhammad, Peter McDonald, David Johnson, Dana Ferraris, Anthony R. Fehr

Coronaviruses (CoVs) are well-known to emerge from zoonotic sources and cause severe human and veterinary diseases, including recent outbreaks of MERS-CoV and SARS-CoV-2. All Coronaviruses (CoVs) encode for macrodomain protein (termed Mac1) that binds to and removes ADP-ribose from protein. Mac1 is essential for CoV pathogenesis, though how it promotes virus replication is still widely unknown. Developing Mac1 inhibitors would be beneficial for identifying the functions of Mac1 and could be used to treat disease caused by CoVs. MHV-JHM is an ideal model for testing Mac1 inhibitors because Mac1 is essential for the replication of this virus. Previous screens identified a compound, MCD-628, that inhibited Mac1 activity in vitro at low micromolar levels. However, MCD-628 had tested compounds with methyl and ethyl esters modifications at the same site, which could be converted back into MCD-628 by cellular esterases. We demonstrated through eADME that the methyl-ester version of MCD-628 had a greater LogD value than MCD-628, indicating that it has higher lipophilicity and likely increased membrane permeability. Importantly, we found that the methyl- and ethyl-esters of MCD-628, and two additional derivates of MCD-628, inhibited MHV replication in cell culture without notable cytotoxicity. We will use additional biological assays to confirm these results and identify drug-resistant mutants, which could indicate potential mechanisms used by Mac1 to inhibit virus replication.

10. The Synthetic Chemical Biology Core (SCB): A Resource For Research In Chemical Biology

Indeewara Munasinghe, Chamani T. Perera

The Synthetic Chemical Biology Core strives to provide comprehensive synthetic chemistry capabilities to investigators under one roof. The synthetic expertise of the core includes but is not limited to, novel and commercially unavailable small molecules, fluorescent molecules, and custom peptides. The core assists in identifying hits for medicinal chemistry optimization in infectious disease targets and provides synthesis capabilities for structure-activity studies of said hits. The core staff will work with investigators to design and synthesize novel molecular probes to facilitate their research. SCB core encompasses the Purification and Analysis Laboratory (PAL) that provides purification, analysis, and quality control of compounds via LC/MS. The SCB core also provides MALDI-TOF analysis of biomolecules.

11. Identification of molecular targets of PICH remodeling activity for faithful chromosome segregation

Bunu Lama, Daniel Keifenheim, Anita Saraf, Victoria Hassebroek, Duncan J Clarke and Yoshiaki Azuma

A broadly utilized therapeutic strategy is targeting of mitosis to selectively kill cancer cells. Because cancers frequently have an elevated frequency of chromosome segregation errors, they are selectively susceptible to further disruption of mitotic processes needed for faithful genome transmission. Plk1-interacting checkpoint helicase (PICH, also known as ERCC6L) has recently emerged as a novel cancer therapeutic target due to having critical roles in mitosis that rely on the ATPase-dependent DNA translocase activity of PICH in many types of cancer overexpressing PICH, including triple-negative breast cancer,colorectal cancer,neuroblastoma. Because PICH's remodeling activity is required for these cancer cells survival, identification of target of PICH activity is critical to advance PICH-targeting therapeutic potential.

We discovered that PICH has ability to bind with SUMOylated proteins on mitotic chromosomes via its SUMO interacting motif(SIM).Conditional PICH depletion and replacement to mutant showed that PICH's translocase activity and SIM are required for preventing chromosome bridge formation and controlling abundance/localization of chromosomal SUMOylated proteins, suggesting PICH's function as SUMOylated protein remodeler is critical for mitosis. Here, we established a method to identify chromosomal SUMOylated proteins which can be targeted by PICH's

remodeling activity. The identified target proteins will be applicable in potential treatment of cancer by targeting PICH function.

12. The Role of PTPN22 and its Autoimmunity Associated Minor Allele during Virus Infection

Alec Bevis, Saiedeh Nasiri, Catherine Kerr, Tony Fehr, Robin Orozco.

The PTPN22 common minor allelic variant, rs2476601, is present in 5-15% of North Americans and is associated with multiple autoimmune diseases. PTPN22 is expressed exclusively in immune cells and regulates numerous immune cell functions including T-cell receptor (TCR) signaling and the selective production of type I interferons. Previous studies show that mice lacking Ptpn22 (PEP-null) or expressing the murine equivalent of the minor allele (PEP-619WW) can clear the chronic virus infection Lymphocytic choriomeningitis virus clone 13 (LCMV-cl13) where wildtype (PEP-WT) mice cannot. This is paired with enhanced T cell and myeloid cell function over PEP-WT animals. However, the mechanisms by which PEP-null and PEP-619WW mice clear chronic virus infection are unknown. Additionally, the cell-autonomous role of Ptpn22 in myeloid cells during virus infection remains unclear. This research aims to interrogate the cell-specific effects accountable for this increased LCMV-cl13 clearance and how the loss of Ptpn22 or its minor allelic variant impacts other virus infections. To determine if dendritic cells as the sole PEP-null cell are sufficient to clear LCMV-cl13 infection, we used a CD11c-Cre Ptpn22 fl/fl conditional knockout mouse. Furthermore, we tested the impact of PEP-null and PEP-619WW during additional RNA virus infections, Vesicular stomatitis virus (VSV) and Mouse Hepatitis Virus (MHV). In these studies, we examine viral tropism, replication, and pathology. Considering PTPN22 regulates a wide range of immune functions, it is vital to better understand the pivotal role this gene may play in the balance between sufficient anti-viral immune responses and overactive responses leading to autoimmunity.

13. Autoimmunity-associated allele of PTPN22 changes Dendritic Cell function during virus infection

Anam F. Shaikh, Alec Bevis, Saeideh Nasiri, Nancy Schwarting, Tammy Cockerham, and Robin C. Orozco

Conventional dendritic cells (cDCs) are elite activators of T cells, which then control virus infection. Altered cDC function causes inferior T cell activation which can result in persistent virus infection. cDCs activate T cells through receptor-ligand interactions and cytokine production and changes in either or both leads to abnormal T cell responses. Previous studies haveestablished that the autoimmunity-associated minor allele of PTPN22, 1858C>T, disrupts cDC cytokine production within autoimmune contexts. PTPN22 is expressed in all hematopoietic cells and regulates Type I IFN production in myeloid cells. Despite the importance of cDCs during virus infection, little is known about how the PTPN22 minor allele impacts cDC anti-viral immune responses. To address this research lacuna, our labemploys mice expressing the murine equivalent of the minor allele (PEP-619WW) and the well-established persistent virus infection model LCMV-cl13. We hypothesize that PEP-619WW impacts DCs in a pleiotropic manner, which leads to improved anti-viral immunity. Our results show that PEP-619WW mice can clear LCMV-cl13 infection, whereas wildtype mice (PEP-WT) cannot. Specifically, PEP-619WW DCs have earlier viral clearance and have a more immunostimulatory phenotype. To elucidate the DC cell-specific effects of Ptpn22 and its minor allele on LCMV-cl13 infection, we measured the infectivity of PEP-WT, PEP-null, and PEP-619WW bone marrow-derived dendritic cells (BMDC). We also evaluated relevant immune inhibitory and stimulatory markers on infected BMDCs. Taken together, this study shows how PEP-619WW changes the DC response to virus infection which may impact the larger anti-viral immune response.

14. Bioactive small molecules produced by the gut commensal Enterocloster citroniae modulate Vibrio cholerae behavior

Saeideh Nasiri, Heidi Peur, and Louis Caetano M. Antunes

Microorganisms living in the human gut have long been known to protect their hosts against pathogens. We previously showed that organic extracts containing small molecules from human feces significantly alter global gene expression in the human pathogen, Vibrio cholerae. In particular, the expression of motility and chemotaxis genes was markedly reduced in cultures grown in the presence of the fecal extract. Here, we focus on one gut microbiota species, Enterocloster citroniae, and demonstrate that extracts from pure cultures of this commensal can also dampen Vibrio cholerae motility. We have also established infection protocols using V. cholerae and A-549 and HT-29 cell lines to investigate the impact of E. citroniae extracts on V. cholerae interactions with host cells. Furthermore, to determine the chemical nature of active compound(s), a series of purification methods culminating with High Performance Liquid Chromatography assays will be adopted. Mass spectrometry and nuclear magnetic resonance (NMR) assays will be performed to determine the chemical composition of active compound(s). To that end, we have used 1H and 13C NMR to profile the chemical composition of extracts from active strains, and common features could be detected. Once compound(s) are purified and the structures are identified, we will investigate the effects of pure compounds on V. cholerae behavior. Together with previous studies, this work will shed light on V. cholerae interactions with its host and theassociated gut microbiota through the lens of chemical biology.

15. Genetic Screens to identify genes acting downstream of Mab-5/Hox in the posterior migration of QL neuroblast in Caenorhabditis elegans

Vedant Jain, Erik A Lundquist

Normal cell migration forms the basis of many developmental processes including the development of the central nervous system. Interference with this process

can lead to a variety of neurological and developmental disorders. The Q neuroblasts as a model in Caenorhabditis elegans are a useful model to study neuroblast migration at a single cell resolution. Q neuroblasts are a pair of bilateral neuroblasts that are born in the posterior-lateral region of the animal. Of this pair, QL is born and resides at the left side, whereas its sister QR is born and resides at the right side of this animal. The migration of these cells is divided into two phases. In the first phase of migration, QR protrudes and migrates anteriorly over V4 seam cell, whereas QL migrates posteriorly over V5 seam cell. Complex genetic interactions involved in the first phase of migration have already been deciphered. The second phase of migration is Wnt dependent and begins after the first phase and the first Q cell division. QL descendants QL.a/p encounter EGL-20/Wnt which is a posteriorly expressed Wnt ligand. This ligand leads to initiation of the canonical Wnt pathway and transcription of a transcription factor called MAB-5/Hox in QL. mab-5 expression in QL.a enables its migration posteriorly over QL.p. After migrating posterior over QL.p it undergoes another set of division to generate two daughter cells QL.aa and QL.ap out of which QL.aa undergoes apoptosis. As for QL.ap, it continues migrating posteriorly in a mab-5 dependent manner and finally differentiates into a sensory neuron called PQR. Mab-5/Hox is both necessary and sufficient for the migration of QL to the posterior of the animal. However, the genes acting downstream of this transcription factor have remained unknown for decades. To answer this question, we carried out single-cell RNA sequencing on early Q cells in mab-5 loss of function mutants and identified genes that were downregulated when compared to WT. Theoretically these genes would be positively regulated by mab-5/Hox. Through our genetic screens, we discovered two candidates that not only interact with the Wnt pathway but are also necessary for the migration of QL in its WT location. We aim to characterize the roles of both these genes in the migration process.

16. Inhibition of HuR/CD147/IL-6 axis enhances anti-PD1 immunotherapy response in cancer

Qi Zhang, Xiaoqing Wu, Liang Xu

Immune checkpoint blockade (ICB) targeting PD-1/PD-L1 has revolutionized cancer treatment. However, durable remission and response rates with ICB monotherapy remain limited. To improve responses, combination therapies have been developed by targeting immune resistance mechanisms observed in 'hot' tumors. Understanding tumor evasion mechanisms could be invaluable for such endeavors.Our study uncovered a novel mechanism that suppresses immune evasion through the HuR/CD147 axis. HuR, an RNA-binding protein overexpressed in various cancers, interacts with oncogenic mRNAs, while CD147, a tumor-associated antigen, is widely expressed in malignancies. We observed downregulation of CD147 in HuR-deficient cells, discovering that HuR regulates CD147 mRNA via binding to its 3' UTR AU-rich elements, as validated by RNP-IP and RNA pulldown assays. KH-39, a HuR-specific small molecule inhibitor, reduced CD147 levels in a dose-/time-dependent manner. HuR inhibition by KH-39 altered immune-related cytokines, including IL-6, known to influence the tumor microenvironment. Combining HuR inhibitor KH-39 with anti-PD-1 antibody in the EMT6 orthotopic mouse breast cancer model enhanced anti-PD1 immunotherapy response and prolonged survival compared to single-agent treatment.

Our findings suggest that targeting the HuR/CD147/IL-6 axis may overcome immune evasion and improve cancer immunotherapy responses. This mechanistic insight holds potential as a promising therapeutic target for clinical cancer therapy.

17. The University of Kansas Protein Structure and X-ray Crystallography Laboratory

Steve Seibold, Lijun Liu, Anne Cooper and Scott Lovell

The Protein Structure and X-ray Crystallography Laboratory at The University of Kansas University (PSXL) collaborates with investigators from various institutions in an effort to obtain the 3-dimensional structures of proteins and small molecules using X-ray crystallography. In addition, the PSXL serves as the main crystallography group for the Seattle Structural Genomics Center for Infectious Diseases (SSGCID) and is responsible for structure determination of various proteins in the pipeline. The capabilities along with examples of structures and ongoing NIH funded projects focused on drug development are presented. These projects highlight the importance of obtaining structural information to provide mechanistic/functional insight for particular proteins and demonstrate the significance of structural biology to facilitate and support drug discovery efforts.

18. Distribution and Features of Beta-Barrels in Bacteria

Daniel Montezano, Rebecca Bernstein, Matthew M. Copeland, Joanna S. G. Slusky

The outer membrane (OM) of Gram-negative bacteria is populated by a functionally diverse group of proteins all assuming the barrel fold. These outer membrane proteins (OMPs) are inserted in the outer membrane with loops exposed on the cell surface, making them essential for interacting with the extracellular environment. Identifying OMPs and understanding how they are distributed across different bacterial groups provides insights about fold and function, and guidelines for design of new barrels for vaccines and novel bioengineering applications. We have previously developed the IsItABarrel algorithm for identifying OMPs and the IsItABarrelDB, the largest and more accurate database of OMPs to date. Here we expanon that work by analyzing the distribution of barrels in bacteria and by analyzing features of barrels for different types and bacterial phyla.

19. Extract of C. parqui reduces the proliferation of triple negative breast cancer cells MDA-MB-231 in vitro

Asbin Bdr. Chand, Roshan Lal Shrestha, Jivan Shakya, Pragati Pradhan, Deena Shrestha, Rajani Malla

There has been a constant demand for targeted therapies against cancers. Most of the anticancer drugs clinically trigger cytotoxic effects through apoptosis, DNA damage, genome instability, and mitotic catastrophe. Nepal's diverse ecological landscape is favorable for many medicinal herbs rich in compounds that may have anti-cancer effects. Thus, we screened selected medicinal plants of Nepal for their anticancer efficacy. The crude methanolic extract was prepared mostly from the dried leaf parts and vacuum evaporated for storage until further experiments. Qualitative phytochemical screening followed by quantitative estimation of total phenolic compounds yielded the highest concentration in Eucalyptus alba bark extract (422.37 ±9.34 mgGAE/gm crude extract).

Similarly, quantitative estimation of total flavonoid content yielded the highest concentration for Ageratina adenophora (156.94±0.7 mg QE/gCE). The anti-inflammatory potential was assessed by DPPH free radical scavenging activity and showed E. alba bark extracts had IC50 values as low as 0.046 mg/mL. Few plant extracts such as Solanum nigrum and Cestrum parqui did not show DPPH radical scavenging activity. However, when tested for anticancer potential by cell proliferation inhibition on triple-negative breast cancer (TNBC) cell line MDA-MB-231, C. parqui extract inhibited cell proliferation by more than 50%. While other plant extracts had a null effect on cell proliferation, extracts from Tinospora cordifolia showed enhanced cell proliferation. In accordance with the cell proliferation inhibition results, C. parqui induced the highest percentage of cell death via apoptosis (68.7%) at 24 hrs which upon prolonged co-incubation proceeded to necrosis. Similarly, E. alba leaf extract also induced apoptosis in approx. 61.7% of the cancer cell during 24hrs co-incubation which proceeded to necrosis upon prolonged co-incubation. We conclude that high flavonoid compound does not correlate with reduced cell proliferation in TNBC cells. Currently, we are investigating the active compound responsible for inducing cell death in C parqui crude extract.

20. MUSASHI1 Affects Intestinal Epithelium Growth By Regulating MTORC1 Signaling

Bikash Pokhrel, Kristi L. Neufeld

The epithelium lining the intestine is the most proliferative tissue in the human body, replacing its entire surface (area roughly that of a tennis court) every 3-4 days. Musashi1(Msi1)protein regulates division and differentiation of stem cells and progenitor cells in a variety of tissues, including the intestinal epithelium. Additionally, regulation of mTORC1 signaling is crucial in maintaining the stem cells at the crypt base of the intestinal epithelium. However, the role of Msi1 in regulation of mTORC1 signaling is unknown.

To study Msi1 function in tissue homeostasis, we generated a transgenic Cre-lox mouse model where tamoxifen administration results in Msi1 over-expression throughout the body. Mice over-expressing Msi1 starting at 4 weeks of age had diminished intestinal epithelial cell proliferation, decreased size of intestinal villi and colon crypts and overall intestinal shortening. We concluded that Msi1 over-expression negatively affects postnatal development and growth of mice. Further, we isolated colon epithelial cells from both Msi1 over-expressing and control mice for bulk RNA sequencing. Gene expression analysis showed downregulation of mTORC1 signaling components (ragd, tm4sf5, LAT4,LAT2). Additionally, gene ontology analysis showed upregulated autophagy processes and downregulated lipid synthesis and transporter activity, consistent with mTORC1 downregulation in Msi1 over-expressing mice.

Our current studies involve identifying and validating direct targets of Msi1 that are involved in mTORC1 signaling and comparing Msi1 regulation of mTORC1 in normal versus cancerous settings. Ultimately, different effects of Msi1 regulation in these two settings could be exploited to find potential targets for colon cancer treatment.

21. Computational Prediction of Chloroplast Outer Envelope β-barrel Proteins

Emily Proctor, Daniel Montezano, Joanna S. G. Slusky

Chloroplasts are plant cell organelles responsible for photosynthesis, which requires a very different set of reactants than glucose-based metabolic systems. These reactions consequentially need different import machineries for different reactants, and the movement of these reactants across the membrane is understood to be accomplished by outer envelope β-barrel proteins. Yet to date, there is not a single chloroplast outer envelope protein that has been structurally solved and we remain unaware of the variety of proteins that participate in outer envelope import and export. Our laboratory has recently developed a computational algorithm, named IsItABarrel, to identify bacterial outer membrane β-barrels. Chloroplast outer envelope β-barrels are likely related to bacterial β-barrels as chloroplasts most likely originated from a primitive prokaryotic cell. We are working to adapt our prokaryotic outer membrane β-barrel identifier for chloroplast outer membrane β-barrels. This will allow us to understand some sequence-based differences between bacterial and chloroplast outer membrane proteins, while also develop a database of chloroplast β-barrels, furthering our understanding of chloroplast biology. So far, we have reimplemented a database of predicted chloroplast β-barrel sequences for all organisms in the domain Eukarya. We present an overview of this database containing 4,812 unique sequences of chloroplast outer envelope proteins. With these sequences, we are able to test and adapt our program for accurately predicting chloroplast outer envelope β-barrels.

22. Computational Identification of Strands in Beta-Barrels using AlphaFold2 Predicted Protein Structures

Tejaswi Nimmagadda, Ryan Feehan, Joanna Slusky

Beta-barrel proteins are located in the outer membrane of bacteria and have functions including

porins, efflux pumps, and enzymes. These beta-barrel-shaped outer membrane proteins are important for research in areas of vaccine development, and antibiotic resistance. Using computational methods to study the structure of these proteins aids in protein design and protein manipulation. The repeating strand-loop-strand-loop topology of beta barrels is often considered a repeat-protein topology. Knowing the number of strands of a barrel helps define the barrel√ïs evolutionary lineage. Here we describe a method of identifying a barrel's strand number from its computationally predicted structure. Our next step is to use this method to 1) identify the lineages of unusual outer membrane proteins and 2) inform generative design of outer membrane proteins.

23. Determining APC's role in UV-induced DNA damage response

Eldric Carreon and Kristi Neufeld

Mutation of Adenomatous Polyposis Coli (APC), is a crucial early step in the progression of ~80% of all colorectal cancers. Our lab has shown that APC, a known cytoplasmic regulator of Wnt signaling, is capable of nucleocytoplasmic shuttling and have identified nuclear roles for APC in Wnt signaling. Other proposed nuclear APC functions include in DNA repair, with APC levels increasing in cells exposed to DNA damaging agents. Additionally, skin fibroblasts isolated from patients with germline APC mutations exhibited heightened sensitivity to UV irradiation, implicating APC in UV-induced DNA damage response. We hypothesized that if APC plays a role in UV-induced DNA damage response, then a loss of APC would lead to an impaired damage response.

Using RKO, a colon carcinoma cell line, with (APC+) and without APC (APC-), we observed that APC- cells displayed an increased sensitivity to varying UV dosages, while APC+ cells showed recovery 24 hours post UV exposure at 10J/M2. Staining with phosphorylated H2AX (p-H2AX), a DNA damage marker, showed APC- cells had a significantly higher amount of p-H2AX in sham irradiated and 10 J/M2 exposed samples as compared to APC+ counterpart. These results highlight a possible function for APC in UV-induced DNA damage response.

24. Functional inhibition of the RNA-binding protein HuR sensitizes triple-negative breast cancer to chemotherapy

Lanjing Wei, Qi Zhang, Cuncong Zhong, Lily He, Yuxia Zhang, Ahlam M. Armaly, Jeffrey Aubé, Danny R. Welch, Liang Xu, Xiaoqing Wu

Chemotherapy remains the standard treatment for triple-negative breast cancer (TNBC), however, chemoresistance compromises its efficacy. The RNA-binding protein Hu antigen R (HuR) could be a potential therapeutic target to enhance the chemotherapy efficacy. HuR is known to mainly stabilize its target mRNAs, and/or promote the translation of encoded proteins, which are implicated in multiple cancer hallmarks, including chemoresistance. In this study, a docetaxel-resistant cell sub-line (231-TR) was established from the human TNBC cell line MDA-MB-231. Both the parental and resistant cell lines exhibited similar sensitivity to the small molecule functional inhibitor of HuR, KH-3. Docetaxel and KH-3 combination therapy synergistically inhibited cell proliferation in TNBC cells and tumor growth in three animal models. KH-3 downregulated the expression levels of HuR targets (e.g., β-Catenin and BCL2) in a time- and dose-dependent manner. Moreover, KH-3 restored docetaxel's effects on activating Caspase-3 and cleaving PARP in 231-TR cells, induced apoptotic cell death, and caused S-phase cell cycle arrest. Together, our findings suggest that HuR is a critical mediator of docetaxel resistance and provide a rationale for combining HuR inhibitors and chemotherapeutic agents to enhance chemotherapy efficacy.

25. Towards a DNA virus model system in Drosophila, Replication dynamics of a DNA virus in insect cell culture

Kent M. Mulkey, Maggie E. Schedl, Robert L. Unckless

Viruses can affect the health and fitness of all forms of life on our planet. Double stranded DNA (dsDNA) viruses such as Herpesviruses and Adenoviruses can cause high morbidity and mortality in humans infected with these viruses. Despite the need for research there are few study systems available to study dsDNA viruses in genetically tractable natural hosts. Therefore, we seek to develop a natural dsDNA virus model in Drosophila so that we can study the host response to a dsDNA virus infection.

Drosophila innubila Nudivirus (DiNV) is a dsDNA virus that naturally infects Drosophila innubila. To be able to work with DiNV in the lab we have developed a cell line from Drosophila innubila embryos called Dinn 3.0 cells. Here, we show the ability of the Dinn 3.0 cells, and other established cell lines, to replicate DiNV. We demonstrate that DiNV grows well in the Dinn 3.0 cells and less so in cells from other fly species. Additionally, we find that DiNV does not grow in cells from other insect species.

Moving forward we will complete studies showing the ability of these cells to make cell free DiNV progeny and once established, this novel dsDNA virus infection model will allow study of the innate immune response of host cells in the context of a natural dsDNA virus infection.

26. Adaptive Mutations in LasR and FusA1 Modulate Ceftazidime Resistance of Pseudomonas aeruginosa

Brielle M. McKee, Rhea Abisado-Duque, Kade Townsend, Kate Woods, Luke Woodward, Josephine R. Chandler

The LasR-I quorum-sensing system contributes to Pseudomonas aeruginosa virulence and increases resistance through unknown mechanisms to antibiotics such as tobramycin and ceftazidime. In prior work, we demonstrated that the effects of LasR on antibiotic resistance could be modulated by a mutation in the elongation factor gene fusA1 (G61A), in which we observed decreased resistance to ceftazidime. Despite the abundant prevalence of fusA1 mutations in chronic infections and their known role in increasing resistance, research regarding their impacts to ceftazidime is limited.

Although ceftazidime's primary mechanism of action is cell-wall synthesis inhibition, the drug's lethality is known to be enhanced by generation of reactive oxygen species (ROS), which cause damage to DNA and other essential processes. The cell manages ROS toxicity, by enacting production of ROS detoxifying enzymes through stress pathways, including the quorum sensing and stringent response networks. We tested the hypothesis that negative effects of the lasR and fusA1 G61A mutation on ROS detoxifying enzymes, cause an accumulation of ROS, which results in increased susceptibility to ceftazidime. In support of this hypothesis, we observed that transcription of the ROS detoxifying enzyme katA is ~5-fold higher than the lasR and fusA1 G61A mutants. By synthetically inducing KatA in the lasR mutant, we observed restored resistance, supporting the idea that LasR protects against ROS-dependent ceftazidime lethality. However, no effect to resistance was observed when KatA was expressed in the fusA1 G61A mutant and current efforts are underway to determine fusA1 G61A's regulatory effects on stringent response and further characterize its role in ceftazidime resistance. In addition, we tested other fusA1 mutations effects on ceftazidime and found mutations (fusA1 T119A and C1446A) that increase resistance to ceftazidime, highlighting the large scale that fusA1 mutations can have in the regulation of antibiotic resistance and the importance of studying their effects. Overall, our results point to a potential mechanism of quorum-sensing regulated ceftazidime resistance through ROS detoxification and furthers the current understanding of the evolution of antibiotic resistance in P. aeruginosa.

27. Determining the Importance of RsbV1's Phosphorylation State for Chlamydia trachomatis' Growth and Progeny Production

Diego Prieto, Lexie Cutter, Scott Hefty

Chlamydia trachomatis is the most prevalent sexually transmitted bacterial infection worldwide, representing a major public health concern. This obligate intracellular bacterium has a phylum-defining biphasic developmental cycle whose regulatory signals and mechanisms are still poorly understood. One protein system that has been shown to govern this cycle's regulation is the Rsb system. This system works based on the phosphorylation state of an intermediate, RsbV1, which determines the activity of a periplasmic sensor phosphatase and a terminal protein partner kinase, RsbU and RsbW respectively. Although genetic disruption of RsbU has been shown to cause a dramatic decrease in progeny production, disruption of RsbW does not, making the Rsb system's role in C. trachomatis' development unclear. The importance of RsbV1's phosphorylation state was examined by studying the effect of its overexpression on progeny production and growth with a tetracycline inducible system. Overexpression of a phosphodeficient mutant for RsbV1 RsbV1S56A, was examined alongside. After an 8-hour, induction period, overexpression of RsbV1 led to a slight decrease in progeny production, whereas there was no significant change due to overexpression of RsbV1S56A. Further studies with different induction periods will be performed to clarify the role of RsbV1 in the system, which could provide better insight on C. trachomatis' development and potential ways to address its public health impact.

28. Development and Disease: Stress Response Effects on Synaptic Loss in C. elegans

Jennifer K. Amrein, Molly B. Massengale, Natasha L. LaGrega, and Brian D. Ackley

Stress is a mechanism by which organisms sense and react to their environment. Organismal stress is a risk factor for tauopathies such as Alzheimer's disease (AD). In AD, synapse degeneration is associated with clinical symptoms, e.g., memory loss, etc., before neurodegeneration. In our Caenorhabditis elegans tauopathy models we have demonstrated transgenic animals exhibit a progressive loss of synapses during aging compared to wildtype. When transgenic animals were fed Providencia rettgeri, a mildly pathogenic bacteria, instead of a normal diet of non-pathogenic E. coli, synaptic loss was delayed or temporarily rescued. Using a comparative RNA-Seq approach we compared animals undergoing synaptic degeneration versus those with rescued degeneration. We observed the primary differences between conditions were remarkably like nematodes overexpressing Jumanji-like histone demethylases. Specifically, animals with synaptic degeneration exhibited upregulation of genes that are downregulated by jmjd-3.1, whereas, rescued animals differentially expressed genes in the same direction. The jmjd-3.1 gene is downstream of the mitochondrial unfolded protein response (mtUPR) and is required for life-span extension in response to organismal stress. When exposed to Juglone, a mitochondrial-reactive oxygen species inducing compound, during early development, both wildtype and transgenic nematodes display fewer synapses at the beginning of adulthood. This suggests that there are thresholds of stress that cause animals to exhibit pre-degenerative like phenotypes. Overall, our results suggest that developmental exposure to ROS-induced or proteotoxic stress changes the probability of neurodegenerative-like outcomes. We are working to better understand effects of timing and dosage and how neurons mechanistically change after stress to affect synaptic integrity.

29. Differential Evolution of Pseudomonas against Ribosome-Targeting Antimicrobial Peptide vs Small Molecule Antibiotic

Kervens Accilien, Robert Unckless

Antibiotics are a powerful tool used to mitigate the effects of pathogen infections on the health and quality of life of human populations. Conventional single-molecule antibiotics work by binding to and inhibiting key bacterial function machinery (e.g. polymerase, topoisomerase, ribosome, etc.); ultimately leading to cell death and population collapse. However, microbe populations are sometimes able to escape extinction through genetic adaptation and become resistant to the antimicrobial(s) to which they are

exposed. The development of new antimicrobials does little to alleviate this problem; once a microbe is exposed to an antimicrobial, resistance follows shortly thereafter. A possible solution to this issue is the development of new classes of antimicrobials against which resistance evolution is harder to achieve. Preliminary studies indicate that resistance against peptide antibiotics fits that bill. In a long-term experimental evolution of a microbe, the upper threshold of resistance is several-fold lower for peptides than single molecules. In this study, we further explore this phenomenon by looking more closely at the differences between peptide-resistant and single-molecule-resistant bacteria. To that end, we selected a set of AMPs and ABXs that target that same biological function (peptide synthesis). We first start by determining the minimum inhibitory concentration of each peptide and single molecule. Next, we generate antimicrobial-resistant populations for each antimicrobial category through experimental evolution. Clonal isolates are taken from those populations to examine selective advantage, fitness costs, cross-resistance, and collateral resistance within and between antimicrobial classes. Finally, resistant isolates were sequenced, and resistance-causing mutations were compared between the two classes of antimicrobials.

30. Structures of TolC Bound to Bacteriocins

S. Jimmy Budiardjo, Emre Firlar, **Andrew Daufel**, Alex Bowman, Jason T. Kaelber, Joanna Slusky

The problem of antibiotic resistance poses a growing threat to human health, and as such necessitates the development of novel strategies to disrupt its various underlying mechanisms. One such mechanism in Escherichia coli involves the trimeric outer membrane porin TolC. TolC plays a central role for multiple bacterial efflux systems (i.e. AcrAB and MacAB) , acting as the terminal pore through which antibiotics and other small molecules are pumped out of the cell. This modularity makes it an attractive target for the design of small molecule/peptide efflux inhibitors. Luckily, nature has provided a framework to probe the binding interactions of TolC via the bacteriocins – small, secreted proteins used to conduct both intra- and inter-species warfare. These proteins, known as colicins in E. coli, consist of an N-terminal translocation domain (T), a receptor binding domain (R), and a C-terminal cytotoxic domain (C). Several of these colicins bind TolC via their T-domains to facilitate the translocation of the toxic C-domain into the periplasmic space. We believe that structural characterization of the binding interactions between TolC and various colicin T-domains will help inform the design of small peptide efflux inhibitors to increase the efficacy of antibiotic treatment. In pursuit of this goal, we partnered with Dr. Emre Firlar and Dr. Jason T. Kaelber at Rutgers University to solve the structures of TolC in complex with the T-domains of Colicin E1 (ColE1), Colicin 5 (Col5), and Colicin E1* (ColE1*) using single-molecule cryo-electron microscopy.

31- Role of specific CDKs in HSV-1 lytic infection

Maxim Rodzkin, Angela Fowler, Heba Mostafa, David Davido

Herpes simplex virus 1 (HSV-1) is a DNA-containing enveloped virus that can cause oral, facial, or genital sores. In certain instances, viral infection results in keratitis and life-threatening encephalitis. Similar to other herpesviruses, HSV-1 has two phases of infection: lytic and latent. One viral protein that regulates the HSV-1 lytic-latent cycle is infected cell protein 0 (ICP0). ICP0 is a phospho-protein that stimulates viral gene expression and replication and is required for efficient reactivation from latency. Notably, ICP0's transactivation activity and its ability to impair the host's antiviral responses require its E3-ubiquitin (Ub) ligase activity. Because HSV-1 is an obligate intracellular pathogen, cellular factors are needed for its replication. Published studies and a high-throughput assay developed in our laboratory indicate the activities of several cellular cyclin-dependent kinases (CDKs) are required for HSV-1 replication and ICP0's transactivator function. To determine which CDKs regulate HSV-1 and ICP0, we tested specific inhibitors of CDK-1, CDK-2, and CDKs-4/6 in viral yield assays in a human cell line using a cycloheximide block and release protocol. We observed that inhibitors of CDK-1 and -2 impaired wild-type virus (ICP0+) replication, whereas an ICP0-null mutant (ICP0-) was impaired by the CDK-2 inhibitor. These results strongly suggest that CDK-1 activity significantly enhances viral replication in an ICP0-dependent manner, whereas CDK-2 appears to act in a predominantly ICP0-independent manner. Future studies will examine how specific CDK inhibitors alter the activities of ICP0 and the HSV-1 lytic-latent cycle.

32. Inhibition of XPO1 in Colorectal Cancer

Andrew Evans, Dan A. Dixon

Colorectal Cancer (CRC) is the second leading cause of cancer death in the U.S. Exportin 1 (XPO1; also known as CRM1) transports proteins from the nucleus to the cytoplasm. XPO1 is known to be overexpressed in multiple cancers, including CRC. The overexpression of XPO1 can lead to the excessive removal of numerous cancer-associated proteins from the nucleus, thus XPO1 can promote tumorigenesis. Selective Inhibitors of Nuclear Export (SINE) compounds are a new class of drugs that inhibit XPO1. Eltanexor is a second-generation SINE compound that is currently in clinical trials for multiple cancer types. Here we propose that XPO1 poses as a strong target for the treatment of CRC. Our results show when you treat the CRC cell line HCT116 cells with SINE compounds, there is a reduction in XPO1 protein. Next, we analyzed Eltanexor's tumor prevention ability in the CRC mouse model, APCmin/+ mice. From this pilot study, we observe a significant ($p<0.01$), ~70% reduction, in intestinal tumor burden. Along with reducing tumor burden, the treated mice showed a significant ($p<0.001$) decrease in the frequency of tumors greater than 1mm in diameter from developing. Together,these results highlight the potential for using XPO1 inhibitors as a chemopreventive agent to inhibit the

development of CRC.

33. OmpA Folding is Complex and Robust
Alex Bowman, Jacqueline Stevens. Joanna Krise, Meghan W. Franklin, and Joanna S.G. Slusky

The structural stability and rigidity of outer membrane beta barrels along with the diverse functional roles played by their extracellular loops makes outer membrane proteins (OMPs) prime candidates as scaffolds for protein design. Several OMPs have been shown to spontaneously insert into membrane vesicles in vitro. We investigated the mechanism of in vitro insertion by evaluating the role of the extracellular loops on the folding pathway of a model OMP, OmpA. By replacing the native extracellular loops of OmpA with short, idealized loop sequences and measuring the folding of these constructs into lipid vesicles, we found that (a) OmpA folding is robust with respect to manipulation of its extracellular loops, (b) the prevailing two-state model of spontaneous OMP insertion into membranes is insufficient to properly describe the folding pathway of OmpA, and (c) the accessibility of intermediate and off-target states is variably impacted by extracellular loop replacement.

34. Using AlphaFold2 to predict RME-8 self-oligomerization
Ryan Feehan, Anne Norris, Barth D. Grant, and Joanna S.G. Slusky

RME-8 is a 260 kDa endocytosis protein that helps regulate recycling and degradation activities. Without an experimentally resolved structure or complex, little is understood about the molecular mechanisms that RME-8 uses to regulate itself. Current reports suggest that RME-8 is inhibited by homo-oligomerization. Using AlphaFold2 (AF2), we provide new support for this model. We used the predicted monomeric structure to identify high-fidelity domains. Using insight from these domains, we were able to create RME-8 complexes for a dimer and tetramer. Finally, recent experimental results further support the predicted protein complexes.

35. Toward a dsDNA Virus Model System in Drosophila: Investigating the Dynamics of the Drosophila innubila Nudivirus Grown for One Year in Cell Culture
Margaret E. Schedl, Kent M. Mulkey, Devon A. DeRaad, Robert L. Unckless

Viruses occupy contrasting roles in our society, either posing a serious challenge to human health, or they can be used as biological control agents. Research on how viruses interact with hosts is urgently needed to prevent viruses from causing significant mortality to humans, and to facilitate the use of viruses to control unwanted crop pests. Model systems, such as Drosophila, have been used for decades to experimentally manipulate viruses and hosts to gain a greater understanding of these host-pathogen interactions. Until recently there were no natural DNA virus models in Drosophila. The Uncklesslab is working to fill this void by developing the Drosophila innubila Nudivirus (DiNV), into a model system for studying DNA viruses in vivo. We have developed a D. innubila cell line that has been infected with DiNV for over 12 months, and continues to produce infectious virions. This cell line that provides a constant source of virus is a significant step toward developing DiNV into a model system. However, to understand the practical relevance of this DiNV infected D. innubila cell line, we need to understand any changes at the DNA sequence level and whether adaptation to cell culture or viral attenuation has occurred. Here, we show the DiNV from the D. innubila cells has evolved from the original inoculum in hundreds of significant changes. Future work aims to understand the titer difference of DiNV across a time course of infection, and to engineer DiNV with a bacterial artificial chromosome to allow genetic manipulation of the virus.

36. The role of nuclear APC in regulating MUC2 expression and colonic inflammation
Anika James, Kristi L. Neufeld

Adenomatous polyposis coli (APC) functions to maintain intestinal homeostasis. Although widely appreciated for cytoplasmic tumor suppressor functions, APC roles in other subcellular compartments, or in inflammation are less defined. To study nuclear APC functions, we previously developed a mouse model with compromised nuclear Apc import. These ApcmNLS/mNLS mice were more susceptible to experimentally induced colitis than their wild-type littermates and displayed lower levels of the RNA encoding mucin-2 (MUC2), the main protein of the intestinal mucus barrier. We hypothesize that nuclear APC promotes gut barrier integrity by regulating MUC2 expression. In cultured human colon cells, we showed a positive regulation of MUC2 RNA level by APC and have used chromatin immunoprecipitation (ChIP) to demonstrate an association of APC with MUC2 DNA. ApcmNLS/mNLS mice displayed significantly thinner colonic mucus layers than wildtype mice and also harbored different bacterial species. Overall, this study provides preliminary evidence that nuclear APC regulates colonic MUC2 expression and the mucus barrier, potentially impacting colonic inflammation and its downstream effects, such as colorectal tumorigenesis.

37. Host Glycan Utilization is Influenced by Multiple Transcription Factors in Enterococcus faecalis
Abdulrahman M. Naeem, Mary E. Galloway, Zakria H. Abdullahi, and Lynn E. Hancock

Enterococcus faecalis is a gram-positive commensal that colonizes the GI tract of most animals including humans. It is also an opportunistic pathogen and causes serious infections including bacteremia and UTI. Many E. faecalis strains also exhibit resistance to many antibiotics. Previous studies demonstrated the ability of E. faecalis to utilize host-derived glycans as a carbon source, and several E. faecalis glycosyl hydrolases have been implicated in utilizing these carbon sources. We focused on identifying regulatory factors that controlexpression of proteins involved in glycan uptake and processing. The promoter regions of three genes

involved in glycan transport (ef2223) and glycan processing by alpha-mannosidases (ef1708, ef2217) were inserted into a luciferase reporter plasmid (pKS320). EF2223 is the first gene of a larger operon (ef2223-21) that encodes a novel ABC transporter. EF1708-07 is a predicted two gene operon encoding a GH38 family glycosyl hydrolase (EF1708), predicted to cleave the α-1,6- N-linked mannose residues, and EF1707 is a predicted GH125 family glycosyl hydrolase that cleaves α-1,3- N-linked mannose residues. EF2217 is GH92 family member shown to cleave the α-1,2- N-linked mannose residues. A luciferase assay was conducted to observe the expression of the target genes. These luciferase reporter plasmids were introduced into the parental V583 strain, as well as ΔccpA, ΔyesN and Δ ef1709 strains to assess the role of these various transcription factors on gene expression with various carbon sources. Results of these experiments show that each transcription factor plays a role in regulating host glycan utilization in E. faecalis.

38. Preclinical Core Facilities

Xiaoqing Wu, Liang Xu

The Experimental and Preclinical Imaging Core provides investigators with the ability to optimize drug treatments in small animals and cell lines, including both small molecule and nanoparticle drug delivery. The capabilities of the near-infrared and bioluminescence imaging services are compatible with most small animal models. These services allow investigators the opportunity for in vivo target validation and dynamic analysis of mechanism of action of novel molecular therapeutics which will advance drug discovery projects more efficiently from bench to bedside. In addition, investigators will learn more about the molecular mechanisms associated with different diseases.

The Biological Irradiation research laboratory provides investigators the ability to examine the effects of radiation treatment upon particular models such as yeast, fruit flies, nematodes, zebra fish, mammalian cells, and small animals. This provides investigators the capability to understand the effect of ionizing radiation on critical cellular responses, such as DNA repair, cellular proliferation and inflammation. In addition, investigators will be able to develop novel therapies using combination treatments of irradiation and small molecules.

39. Engineering a GFP-Mimetic Outer Membrane Protein through Rational Design and Deep Learning

Rik Dhar, Ryan Feehan, Meghan Franklin, Joanna Slusky

The beta-barrel protein structure is typically found in the outer membrane proteins of Gram-negative bacteria, mitochondria, and chloroplasts. While the beta-barrel fold is distinct from most membrane proteins composed of transmembrane helices, it is not unique to only membrane proteins. Green fluorescent protein (GFP), a cornerstone of fluorescence-based biological research, shows remarkable structural similarity to membrane beta-barrel proteins. The structure of GFP is composed of an 11-stranded beta-barrel that wraps around an alpha-helical core. This alpha helix contains the amino acids that form the fluorescent chromophore through autocatalysis. As there is no known naturally occurring fluorescent outer membrane protein, we use rational design in combination with a protein design method based on deep learning to engineer a GFP-mimetic outer membrane protein (Gmim). We selected an outer membrane autotransporter protein, intimin, that consists of a 12-stranded beta-barrel that wraps around a disordered helical structure, as a scaffold for our design. We replace the disordered helical structure of intimin with the chromophore-forming region of GFP. This rationally designed structure is then further refined using the deep learning method proteinMPNN. We report here some preliminary results on two Gmim design variants that we selected for experimental testing. These designed fluorescent membrane proteins offer promising applications in several areas, including microscopy imaging for visualization of membrane dynamics, for the study of membrane protein interactions, and use as biosensors for detecting changes in the local membrane environment.

40. Targeting EWS-dependent pathways as chemotherapeutic vulnerabilities in Ewing sarcoma

Evan Schulz, Mizuki Azuma, Haeyoung Kim

Ewing sarcoma (ES) is the second most common adolescent bone cancer, and currently, it has no available targeted chemotherapies. To develop targeted chemotherapies, the role of ES-specific events in tumor chemosensitivities must be understood. As the hallmark of ES is the t(11:22) chromosomal translocation that generates EWS/FLI1 expression and EWS haploinsufficiency, we hypothesized that the loss of EWS functionality derived from EWS/FLI1 inhibition of EWS and EWS haploinsufficiency sensitizes cells to drugs targeting EWS-dependent processes. Since EWS functionality is required for proper mitotic Aurora B (AuB) activity and spindle formation, we investigated whether EWS/FLI1 expression and EWS haploinsufficiency sensitize cells to microtubule disruptors (MtD) or AuB inhibitors (AuBi). Greater Trypan blue and Annexin V staining under drug treatment is interpreted as more sensitive drug response. Comparing A673 ES cells to DLD-1 colorectal carcinoma and mesenchymal stems cells (MSC) under either nocodazole (MtD) or ZM447439 (AuBi) treatment, A673 cells are less viable with more apoptotic activity than DLD-1s and MSCs. Using a DLD-1 cell line engineered with inducible EWS/FLI1 expression and EWS haploinsufficiency, we compared drug responses across all combinations of inducible EWS/FLI1 expression or EWS haploinsufficiency. Under nocodazole treatment, cells with induced EWS/FLI1 expression and EWS haploinsufficiency are significantly less viable ($p<0.01$) and have more apoptotic activity ($p<0.05$) than uninduced cells. These results indicate EWS/FLI1 expression and EWS haploinsufficiency sensitize cells to MtD by nocodazole. Taken together, ES may be targetable through EWS-dependent pathways, exploiting loss of EWS functionality (derived from EWS/FLI1 and EWS haploinsufficiency) as a tumor-specific chemotherapeutic

vulnerability.

41. Quorum sensing control of aminoglycoside antibiotic resistance in Pseudomonas aeruginosa

A.S. Brown, R. G. Abisado, J. H. Kimbrough, N. E. Smalley, B.M. Mckee, V.D. Craddock, A. A. Dandekar, and J. R. Chandler

Pseudomonas aeruginosa is a leading cause of hospital-acquired infections and the primary cause of mortality in cystic fibrosis patients. It's also a model for studying quorum sensing (QS), which uses chemical signals to coordinate behavioral changes in response to population density. The LasR protein is a major transcriptional activator in the P. aeruginosa lasR-lasI quorum-sensing (QS) system, controls production of toxins and secreted proteases (public goods) and has a central role in the activation of many virulence genes. LasR also regulates resistance to aminoglycoside antibiotics (e.g. gentamicin and tobramycin). Bizarrely, lasR deficient mutants are commonly found in clinical samples from patients treated with antibiotics. It is unclear whether these mutants are products of environmental selection, if they adopt the role of social cheaters and exploit cooperator production of public goods, or if the population develops LasR mutations during adaptation that alter the antibiotic susceptibility of the lasR mutants. We previously generated and characterized P. aeruginosa populations passaged on tobramycin and showed that mutations accumulate in these populations that inactivate ptsP (phosphoenolpyruvate-protein phosphotransferase gene). Inactivating ptsP increases antibiotic resistance, quorum-sensing activity, pyocyanin production, and policing of lasR cheaters. Our current research shows that gentamicin triggers a different evolutionary trajectory that leads to the accumulation of mutations that enhance LasR activity and cheater suppression through ptsP-dependent and independent mechanisms. Some of the gentamicin adapted variants along with naturally emergent lasR variants show enhanced antibiotic resistance when LasR is inactivated. Our work focuses on understanding the link between antibiotic resistance and QS in P. aeruginosa and examines what roll antibiotic therapy plays in the evolutionary trajectory of clinical infections. Results of these studies could aid in developing novel therapies exploiting P. aeruginosa's evolutionary biology during infection.

42. Ironing Out a Role for HAF-6 in C. elegans

Sutton Stegman, Sudeep Shakya, Mara Stout, Mariana Ochao, Robb Gibbs, Dr. Lisa Timmons

Iron is an essential dietary component for all cells and organisms. Iron is required for essential enzymatic processes, yet because iron can easily gain and lose electrons, iron can also cause oxidative damage, leading to genomic instability and cell death. Not surprisingly, cells have adapted remarkable molecular strategies for storage and trafficking of iron in such a manner that oxidative damage is avoided and iron homeostasis is achieved. Inter-cellular iron trafficking is well regulated, with ferroportin (FPN) being the sole exporter of iron from intestinal stores. Iron is also trafficked intra-cellularly by several proteins, including subclasses of ABC transporters; however, little is known regarding precise roles of ABC transporters in iron trafficking. Here we reveal that the HAF-6 ABC transporter functions as a homodimer and transports iron substrates. Additionally we show that a mutation in a highly conserved region of C. elegans fpn-1.1 render C. elegans more sensitive to iron deprivation compared to wild type. This observation is rescued when our fpn-1.1 mutant strain also has a mutation in the ABC transporter haf-6. The amino acid sequence of which has extensive similarity to human ABCB8. The human ABC transporter ABCB8 localizes to the inner mitochondrial membrane where it plays a role in mitochondrial iron homeostasis and the maturation of cytosolic Fe-S proteins. Our studies not only help ascribe a function to an orphan ABC transporter in C. elegans, but also help elucidate common roles for the conserved iron-trafficking molecules FPN and ABCB8, proteins which are stress responsive in both C. elegans and humans. Our findings may help elucidate functions of these proteins in anemia and haemochromatosis, and perhaps also in anemia of inflammation.

43. Developing a Novel Assay for Transposon Activity and the Influence of Iron Trafficking

Mariana Ochoa, Brooke Petersen, Mara Stout, Lisa Timmons

Transposons are segments of foreign DNA characterized by their ability to move within a genome. When a transposon expresses its transposase enzyme, the transposon sequence can move to a different location within the genome. This is a stressor to cells because reinsertion of transposons is not limited to a specific location. As a result, transposons can insert within a gene, resulting in loss-of-function mutations. The cell uses RNA interference (RNAi) as one tool to protect itself from activation of transposons and potential transposon insertion mutations. RNAi describes not only this cellular mechanism that silences genes and influences gene expression, but also describes a technique that allows scientists to silence genes using double stranded RNA to trigger the RNAi response. Interestingly, we isolated two mutations in genes, rde-1 and rde10, required for RNAi that are caused by insertion of a transposon in Caenorhabditis elegans. These transposon insertion alleles cause the resulting C. elegans strains to behave as RNAidefective mutants as they fail to respond to experimental delivery of double stranded RNA. We first determined that the transposons inserted into rde-1 and rde-10 are capable of mobilizing. Our second goal follows up on the observation that transposons mobilize in strains that have a haf-6 mutant background, but not in a haf-6 fpn-1.1 double mutant background. The haf-6 gene encodes a transmembrane ABC transporter protein that is involved in iron trafficking. The fpn-1.1 gene also encodes an iron trafficking transmembrane protein that releases iron from intestinal stores to the circulating fluids. We developed a novel transposon mobilization assay using our recently isolated transposon insertion

allele of rde-1 and performed genetic crosses to generate marked strains that will help elucidate potential roles of iron trafficking to protect the germline from transposon activation.

44. Horizontally transmitted avirulent Nora virus is associated with upregulation of canonical immune pathways in cells of the fat body.

Nilanjan Roy, Robert L. Unckless

Viruses are ubiquitous and can spread vertically through the embryo or in early-stage larvae. If virulence is low, these viruses can go undetected and can build up persistent infections. In many Drosophila studies, researchers are unaware or ambivalent about the fact that the flies may be infected with vertically transmitted avirulent viruses, and these viruses can affect the canonical gene regulation systems in Drosophila. Through viral genome mapping, we have found a vertically transmitted avirulent virus called Nora virus in a published Drosophila fat body single-cell RNA sequencing (scRNA) experiment. Nora virus is a single-stranded RNA virus. Recent studies have shown Nora virus often builds up persistent non-pathogenic infection but if the expression of the virus increases then it can decrease the lifespan of the flies. We wanted to find out how this virus can influence the major innate immune systems of Drosophila using this published fat body scRNA dataset. The four canonical immune pathways of Drosophila that are known for innate immune response to pathogens are Toll, IMD, RNAi and JAK-STAT pathways. Our results show specific immune genes of these pathways are affected by Nora virus leading to upregulation of Toll & IMD pathways and downregulation of RNAi and JAK-STAT pathways. Other pathogen defense pathways also showed response and expression change in Nora virus infection as well. This study aims to provide a complete immune profiling of Drosophila in Nora virus infection at single cell resolution.

45. Investigation of In Vivo Dimerization and Subcellular Localization of HAF-6 in C. Elegans

Maximino Emerson, Lisa Timmons

As one of the largest superfamilies of proteins in all of biology, ABC transporters play important roles in cellular uptake, cellular export, and both intercellular and intracellular trafficking of small molecules, referred to as "substrates"across all cellular membranes. Currently, the understanding of ABC transporter function is limited by the relatively small number of identified substrates. The Caenorhabditis elegans ABC transporter gene haf-6 was identified for its influence on gene silencing mechanisms in this organism. The substrate(s) of the HAF-6 protein are unknown; furthermore, the HAF-6 protein is a half-molecule ABC transporter and must dimerize in order to function. Theoretically, HAF-6 could dimerize with any of the products of the nine half transporter genes in the B subfamily of C. elegans ABC transporters. Using both GFP reporters and immunofluorescence staining of fixed tissue, HAF-6 has sporadically displayed subcellular localization patterns around the nuclear periphery rather than its typical localization in the ER. Localization to the nuclear periphery is rarely described for ABC transporters in normal tissue. This type of pattern is seen in a variety of cancer cells, particularly those which are highly aggressive and drug resistant. In the pursuit of fully elucidating functional roles for HAF-6 and investigating the implications of ABC transporter localization to the nuclear periphery, we must first understand the structure of the functional transporter complex such that we may identify substrates which it traffics through biochemical assay. Here, I describe a split-GFP approach to test for dimerization partners of HAF-6 and the progress-to-date in identifying its partners.

46. Induction of pilus gene cluster involved in interspecies interactions by acyl-homoserine lactone-dependent eavesdropping in Chromobacterium subtsugae

Nathan Smith, Pratik Koirala, Josephine Chandler

Many proteobacteria regulate gene expression in a population density-dependent manner using "quorum sensing," a cell-cell signaling system involving acyl-homoserine lactone signals (AHLs). AHL signal synthase and receptor pairs are thought to co-evolve such that the AHL receptor is activated only by "self" or highly related bacteria. While some AHL receptors are highly specific, many AHL receptors have more relaxed specificity or "promiscuous" AHL response. Here, we use the soil saprophyte Chromobacterium subtsugae as a model for understanding non-self AHL-dependent gene regulation. C. subtsugae has a single AHL circuit, CviI-R. CviI produces N-hexanoyl-HSL (C6-HSL), and CviR responds to C6-HSL and a variety of other AHLs, including C8-HSL. Using RNAseq we determined the set of genes responsive to C6-HSL, C8-HSL or both signals. We found that a subset of genes responsive to C6-HSL are also responsive to C8-HSL. Many of these are antibiotic biosynthesis genes, suggesting the induction of these genes by non-self AHLs might be important for competing with other bacteria in a polymicrobial community. Surprisingly, about 30 C. subtsugae genes respond only to C8-HSL, and not to C6-HSL. Among these are genes predicted to code for conjugative functions, motility, DNA uptake and nutrient acquisition. Future experiments are aimed at testing the function of these genes and testing the mechanism of their regulation by C8-HSL. Our results suggest that promiscuous AHL signal receptors may have specific roles in sensing and responding to non-self populations and support the idea that promiscuity may have an adapted benefit to bacteria.