Department of Molecular Biosciences
College of Liberal Arts and Sciences

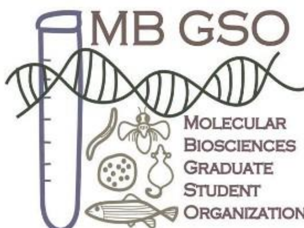
Molecular Biosciences Symposium 2024

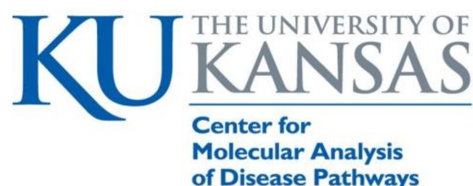
August 23, 2024

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

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

THE UNIVERSITY OF KANSAS
CANCER CENTER

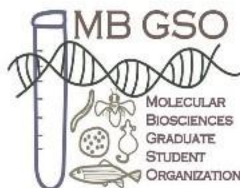




2024 Molecular Biosciences Departmental Symposium

Aug. 23, 2024 – Burge Union Forum CD

OPENING ACTIVITIES	
8:30 AM	Poster Set-up & Refreshments
9:00 AM	Welcome
FACULTY TALKS	
9:10 AM	Dr. Yoshiaki Azuma Molecular functions of TopoIIa on accurate chromosome segregation to maintain integrity of genome.
9:40 AM	Dr. David Davido From Structural to Chemical Biology: Understanding Herpes Simplex Virus-Host Interactions.
10:10 AM	Dr. Robin Orozco Uncovering the role of PTPN22 during virus infection.
10:40 AM	Break
KEYNOTE SPEAKER	
10:50 AM	Dr. Stephen McSorley, UC Davis School of Veterinary Medicine Making Good Memories: Understanding protective immunity to bacterial infections
11:50 AM	Announcement of Mark Richter Memorial Plaque and Award
11:55 AM	Lunch
STUDENT TALKS	
1:00 PM	Andrew Evans XPO1 inhibition modulates the Wnt/β-catenin signaling pathway to reduce colorectal cancer tumorigenesis.
1:20 PM	Bunu Lama PICH remodels SUMOylated protein on mitotic chromosomes to control Spindle Assembly Checkpoint.
1:40 PM	Bikash Pokhrel Musashi1 regulates mTORC1 pathway, potentially controlling the growth of the intestinal epithelium.
2:00 PM	Nilanjan Roy Persistent Viral Infection Elicits Distinct Gene Expression Profiles in Laboratory *Drosophila* Fly Lines.
2:20 PM	Break
POSTER SESSIONS	
2:30 – 3:15 PM	Poster Session I (Even)
3:15 – 4:00 PM	Poster Session II (Odd)
PICNIC & SOCIAL	
5:30 PM	Picnic & Social



THE UNIVERSITY OF KANSAS

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Keynote Speaker

Making Good Memories: Understanding protective immunity to bacterial infections

Stephen McSorley Ph.D.,
Professor, Dept. of Anatomy, Physiology, & Cell Biology,
Veterinary Medicine,
University of California Davis

Systemic *Salmonella* infections are responsible for almost 1 million deaths annually and there is an urgent need to understand the mechanisms of protective immunity and develop new vaccines. An effective *Chlamydia* vaccine is also needed, given the rising incidence of *Chlamydia* infection and the potential for serious reproductive harm in otherwise healthy young women. One commonality between these two infections is the requirement for CD4 T cells to provide protective immunity. Understanding the antigens targeted by protective *Salmonella*-, and *Chlamydia*-specific CD4 T cells is critical for vaccine development. Equally important is developing a deeper understanding of the effector mechanisms and circulation patterns of protective T cells in these infections. Work from mouse models has shed light on the commonalities and stark differences between host response to these infections and will hopefully form the groundwork for effective vaccines.

Faculty Talks

Molecular functions of TopoIIα on accurate chromosome segregation to maintain integrity of genome

Yoshiaki Azuma
Department of Molecular Biosciences, University of Kansas, Lawrence, KS 66045

Chromosome segregation during mitosis is a fundamental process in which the replicated genome is evenly inherited by two daughter cells. This genome transmission process is coordinated with both dynamic structural organization of the chromosomes, including condensation of chromosomes, resolution of cohesin-mediated sister chromatid cohesion, and DNA-entanglement resolution, and activity of chromosome motor machinery composed by kinetochore attached microtubule and motor proteins. Structural organization of chromosomes and timing of motor activity needs to be orchestrated to faithful separation of sister chromatids. DNA Topoisomerase II (TopoII) is known for its essential role in these processes as a structural component of mitotic chromosomes as well as an enzyme that resolves entangled genomic DNA. In vertebrate, TopoIIα isoform uniquely performs mitotic functions, which requires its C-terminal domain (CTD) that is distinct from TopoIIβ isoform and dispensable for enzyme (decatenase) activity. This presentation, I will discuss our recent findings of specific molecular mechanisms of TopoIIα-CTD for accurate chromosome segregation by controlling timing of mitosis and regulating chromosomal structural organization.

From Structural to Chemical Biology: Understanding Herpes Simplex Virus-Host Interactions

David Davido
Department of Molecular Biosciences, University of Kansas, Lawrence, KS 66045

Infection by herpes simplex virus type 1 (HSV-1) can result in a wide range of diseases in humans, ranging from cold sores to blinding ocular infections and encephalitis. HSV-1 has two distinct infections in its viral life cycle: lytic and latent. A lytic infection has high levels of viral gene expression and progeny production, whereas a latent infection results in a lack of infectious virus. As viruses are obligate pathogens, my lab is interested in understanding how virus-host interactions regulate the HSV-1 life cycle, utilizing approaches in structural and chemical biology. First focusing on the viral E3 ubiquitin ligase, ICP0, we wanted to determine how its dimer structure influenced its functions. Notably, ICP0's dimer domain is associated with its transactivation activity and efficient HSV-1 replication. X-ray crystallography studies showed that ICP0's dimerization region generates two β-barrel-like motifs. Computational analyses reveal that ICP0 can either form a dimer or bind to the cellular protein, SUMO-1, via its C-terminal SUMO-interacting motifs but not both. To identify novel host factors required for viral replication, we used a high-throughput cell-based assay to screen several chemical libraries to discover new inhibitors against herpes simplex virus 1 (HSV-1). From this screen, we identified three aurora kinase inhibitors that potently reduced viral gene expression. These inhibitors significantly reduced viral transcript and protein levels and HSV-1 titers at low micromolar concentrations. Collectively, these two approaches indicate viral (i.e., ICP0) and host (i.e., aurora kinases) determinants play pivotal roles in HSV-1 productive infection.

Uncovering the role of PTPN22 during virus infection

Robin Orozco
Department of Molecular Biosciences, University of Kansas, Lawrence, KS 66045

Allelic variation within immune regulatory genes can change the strength and target of the immune response during disease. Autoimmune diseases have complex etiology involving the interplay between genetics and the environment. The allelic variants which predispose individuals to any one autoimmune disease (e.g. Type I Diabetes) are often a risk factor for other autoimmune diseases. One such allele is the Protein Tyrosine Phosphatase Non-Receptor 22 (PTPN22), 1858C>T (rs2476601) allele, which is present in 5-10% of the North American population and is the highest non-HLA risk allele for developing numerous autoimmune diseases. This shared genetic risk combined with the high frequency in the population raises the questions: did the autoimmunity-associated PTPN22 allelic variant evolve for a beneficial, reason? If there is a benefit, could it be harnessed for improved immune-based therapies? Our lab hypothesizes yes to both questions. To address this, we study how this autoimmunity-associated 1858C>T PTPN22 allele regulates the immune response in non-autoimmune disease contexts, such as virus infection. Through this approach we expect to define new ways that PTPN22 and its 1858C>T allele either protects or progresses disease. During my talk, I will review our studies demonstrating a protective role for the PTPN22 1858C>T allele during certain virus infections including a murine model of coronavirus, mouse hepatitis virus.

Student Talks

XPO1 inhibition modulates the Wnt/β-catenin signaling pathway to reduce colorectal cancer tumorigenesis

Andrew Evans[1], Dan A. Dixon[1,2]
[1]Department of Molecular Biosciences, University of Kansas, Lawrence, KS
[2]University of Kansas Cancer Center, Kansas City, KS

Colorectal Cancer (CRC) is the second leading cause of cancer-related death in the U.S. A subset of individuals faces a notably higher likelihood of developing CRC within their lifetime. Hence, there is a compelling need for innovative chemopreventive treatments aimed at minimizing CRC tumorigenesis. Exportin 1 (XPO1; also referred to as CRM1) plays a pivotal role in transporting proteins from the nucleus to the cytoplasm. Numerous cancers overexpress XPO1, including CRC. Selective Inhibitors of Nuclear Export (SINE) compounds, such as Eltanexor, have been developed to target XPO1 and show promising therapeutic benefits. This research evaluates Eltanexor as a chemopreventive agent for CRC. Our findings indicate that in CRC, Eltanexor treatment impairs COX-2 expression by reducing Wnt/β-catenin signaling. Drug sensitivity assays using organoids from Apcmin/+ (a genetic CRC mouse model) tumors and wild-type mouse small intestine tissue show that Apcmin/+ tumoroids are more sensitive to Eltanexor than wild-type organoids. Additionally, when Apcmin/+ mice receive Eltanexor treatment, it significantly reduces tumor burden by approximately 3-fold and decreases the number of tumors larger than 1mm. Collectively, these findings highlight XPO1 as a potent target for CRC chemoprevention.

PICH remodels SUMOylated protein on mitotic chromosomes to control Spindle Assembly Checkpoint

Bunu Lama[1], Yoshiaki Azuma[1]
[1]Department of Molecular Biosciences, University of Kansas, Lawrence, KS

Post-translational modification by SUMO, SUMOylation, is required for maintenance of genome integrity. Either inhibiting SUMOylation or stabilizing SUMOylation in mitosis causes defects in chromosome segregation, hence dynamic SUMOylation of mitotic proteins is critical for faithful chromosome segregation. SUMOylation of mitotic chromosomal proteins recruits proteins with SUMO-interacting motifs (SIM) to specific chromosomal locations in a temporally controlled manner. Polo-like kinase 1-interacting checkpoint helicase (PICH), which is a mitotic chromatin remodeling enzyme, interacts with SUMOylated chromosomal proteins via three SIMs. Defects in both the DNA translocation activity of PICH and its ability to interact with SUMOylated proteins results in chromosome bridge formation in anaphase, suggesting that PICH's remodeling function toward SUMOylated chromosomal proteins contributes to faithful chromosome segregation. Using cell lines that can conditionally deplete endogenous PICH and replace it with PICH mutants, we revealed the phenotypic defects associated with compromised PICH functions. PICH mutants defective in SUMO binding or DNA translocase activity delayed mitotic progression while maintaining Spindle Assembly Checkpoint (SAC) activation, indicated by extended duration of Mad1 foci at centromeres. To gain insight into how PICH's activity towards SUMOylated proteins is involved in sustained SAC activation, we established conditional PICH-depletion/replacement cell lines with endogenous SUMO2 fused to a hexa histidine-tag for isolation of chromosomal SUMOylated proteins. Identification of chromosomal SUMOylated proteins whose abundance on chromosomes is altered in PICH mutants provided candidate proteins to explain the SAC activation phenotype. These included SAC mediator proteins as well as centromere/kinetochore components. Further exploration of each protein's function will enhance our understanding of how mitotic chromosomal SUMOylation can regulate mitosis.

Musashi1 regulates mTORC1 pathway, potentially controlling the growth of the intestinal epithelium

Bikash Pokhrel[1], Kristi L. Neufeld[1]
[1]Department of Molecular Biosciences, University of Kansas, Lawrence, KS

Colorectal cancer (CRC) is the second leading cause of cancer-related death, affecting 1 in every 24 people. CRC develops due to an imbalance in cell division, differentiation, and death in the epithelial cells lining the intestine. Musashi1, an RNA-binding protein, is exclusively expressed in stem and dividing cells and is essential for their maintenance and fate. Our published research showed that ubiquitous overexpression of Musashi1 in mice resulted in reduced growth of the organism and various organs. We employed bulk RNA sequencing and in-vitro studies to determine the mechanism underlying this growth defect. Our studies revealed that Musashi1 inhibits growth by regulating the mTORC1 pathway. The mTORC1 pathway is central to cellular growth, as it controls both anabolic processes like protein and lipid synthesis and catabolic processes like autophagy. Immunofluorescence studies revealed that Musashi1 protein and mTORC1 activity are mutually exclusive in cells of the intestine, consistent with a key role for this interaction in determining the fate of stem and dividing cells. Our current work aims to identify the RNA targets of Musashi1 that regulate the mTORC1 pathway. Given that mTORC1 and Musashi1 are often dysregulated in CRC, our study may lead to improved treatment strategies.

Persistent Viral Infection Elicits Distinct Gene Expression Profiles in Laboratory *Drosophila* Fly Lines

Nilanjan Roy[1], Robert L. Unckless[1]
[1]Department of Molecular Biosciences, University of Kansas, Lawrence, KS

Viruses are ubiquitous and can spread in two main ways: vertically, which involves transmission through embryos or early-stage larvae, and horizontally, which occurs through direct contact, airborne transmission, or indirect contact. If virulence is low, these viruses can go undetected and build up persistent infections which can show little to no effect on the host phenotypes. In many *Drosophila* studies, researchers are unaware or ambivalent about the fact that the flies may be infected with persistent viruses. Through viral genome mapping, we have found Nora virus, *Drosophila* A virus, Newfield virus, *Drosophila* C virus, and Thika virus expression in several published *Drosophila* single-cell RNA sequencing (scRNA) experiments. These viruses naturally infect *Drosophila* in their wild habitats, and now they are observed to be persistently infecting laboratory fly lines too. Although they exhibit no apparent phenotypic effects in laboratory fly samples, recent studies suggest that an increase in the viral titer of Nora virus (single-stranded RNA virus), is associated with a decrease in the lifespan of flies. The primary research question of this study revolves around elucidating the impact of these viruses on the canonical gene regulation systems in *Drosophila*. To do so, we leveraged publicly available *Drosophila* single-cell RNA sequencing (scRNA) data and uncovered significant influences of these persistent virus infections on the major innate immune systems and transposable elements. This study aims to provide a complete transcriptomic profiling of *Drosophila* in persistent virus infection at single-cell resolution.

Poster Session Roster

Poster Session I (2:30-3:15 PM): Even number posters
Poster Session II (3:15-4:00 PM): Odd number posters

No.	Presenter	Title
1	**Abdulrahman M. Naeem**	The three-component signal transduction system YesLMN of Enterococcus faecalis senses host glycans to activate expression of an ABC transporter required for host glycan import
2	**Alec M. Bevis**	The Autoimmunity-Associated Minor Allele of PTPN22 Enhances Antiviral Immunity During Coronavirus Infection
3	**Alfred Buabeng**	EV as a Prognostic Biomarker for Molecular Therapy Targeting RNA-binding Protein HuR in Cancer Immunotherapy
4	**Anam F. Shaikh**	Improving immunity against chronic virus infection using an autoimmunity associated Improving immunity against chronic virus infection using an autoimmunity associated Ptpn22 allele.
5	**Andrew Daufel**	Characterization of TolC Colicin Interactions
6	**Andrew Evans**	XPO1 inhibition modulates the Wnt/β-catenin signaling pathway to reduce colorectal cancer tumorigenesis
7	**Anika James**	Nuclear APC can regulate MUC2 expression and colonic inflammation
8	**Anna Ferkul**	PARP14 Restricts HSV-1 Lytic Replication
9	**Anuradha Roy**	Infectious Disease Assay Development Core: High Throughput Screening Laboratory at the University of Kansas
10	**Asbin Chand**	Role of Haspin kinase in Topoisomerase II Response Checkpoint (TRC) activation in Human Cells
11	**Bikash Pokhrel**	Musashi1 regulates mTORC1 pathway, potentially controlling the growth of the intestinal epithelium
12	**Bren Den Ng**	Characterizing C. acnes and Staphylococcus spp. in polymicrobial biofilms
13	**Brielle M. McKee**	LasR and FusA1-regulated ceftazidime resistance is dependent on stringent response in Pseudomonas aeruginosa
14	**Chamani T. Perera**	The Synthetic Chemical Biology Core (SCB): A Resource for Research in Chemical Biology
15	**CJ Gormly**	The core programmed cell death pathway regulates neuronal migration downstream of the MAB-5/Hox transcription factor in C. elegans
16	**Coral Y. Zhou**	Mechanisms of genome scaling during embryogenesis and evolution
17	**David K Johnson**	Computational Chemical Biology Core: A Chemical Biology of Infectious Disease COBRE Core Laboratory
18	**Eldric Carreon**	Determining a roll for Nuclear APC in UV-Induced DNA Damage Repair
19	**Elle Saenjamsai**	PARP14 in Host-Virus Interactions: Identifying Domains Required for IFN-I Induction and Viral Replication Control
20	**Eryk Yarkosky**	Transcription patterns of quorum-sensing regulated genes in Chromobacterium subtsugae differ under induction by native and competitor signals
21	**Ethan Rogers**	RNA-RNA Interaction of a non-coding region in Hepatitis C Virus Genome Promotes Translation
22	**Evan Schulz**	Mitotic activities of Ewing sarcoma proteins

23	**Felipe L. Teixeira**	Transcriptomic differentiation map of a C. elegans neuroblast lineage at single-cell resolution
24	**Chukwuma Great Udensi**	Bioactive small molecules from the human gut microbiome modulate Salmonella enterica invasion gene expression
25	**Hans M. Dalton**	Dalton Lab Overview: Rare Diseases, Drug Screens, and Essential Genes
26	**Harsha Hapugaswatta**	Role of EWSR1 allele loss and EWSR1-FLI1 fusion in aneuploidy induction of Ewing sarcoma
27	**Jennifer L. Hackett**	Next Generation Sequencing at KU Genome Sequencing Core
28	**Joseph J. O'Connor**	Mutations affecting different biochemical functions of the coronavirus macrodomain, Mac1, indicate that it promotes multiple stages of the viral replication cycle
29	**Kent M. Mulkey**	A *Drosophila* DNA Virus Model
30	**Kervens Accilien**	Differential Evolution of Pseudomonas fluorescens against Ribosome-Targeting Peptide vs Small Molecule Antimicrobials
31	**Kiana Hajiarbabi**	Staphylococcus epidermidis-diffusible molecules inhibit Staphylococcus aureus virulence
32	**Macie Proctor-Roser**	The Impact of PTPN22 on the B Cell Landscape During Viral Infection
33	**Maxim Rodzkin**	Inhibitors of CDK-1 and CDK-2 Diminish HSV-1 Immediate-Early Gene Expression
34	**Ngoc Huan Nguyen**	HuR Regulates the cGAS-STING Pathway: A Potential Therapeutic Target in Cancer Immunotherapy
35	**Parker Sperstad**	Insights into the Isoenergetic Monomeric Structures of the Hepatitis C Virus 3'X RNA
36	**Peter R. McDonald**	Flow Cytometry Core: A Chemical Biology of Infectious Disease COBRE Core Laboratory
37	**Peter R. McDonald**	University of Kansas Biomedical Research Core Facilities
38	**Rayssa Durães Lima**	Cutibacterium acnes Secretes Molecules that inhibit Staphylococcus lugdunensis biofilm formation, adhesion and invasion to human epithelial cells
39	**Regan Krueger**	Determining the Functions of Topoisomerase II as an Enzyme and as a Structural Component on Mitotic Chromosomes
40	**Ryan Grigsby**	Equipment and Services of the Kansas University Nanofabrication Facility
41	**Saeideh Nasiri**	Bioactive Small Molecules Produced by The Gut Commensal Enterocloster citroniae Modulate Vibrio cholerae Behavior
42	**Samalee Banerjee**	Quorum Sensing Regulation by the ptsO and ptsN genes of the Nitrogen phosphotransferase system
43	**Samuel Lim**	Integrating LigandMPNN into Rosetta
44	**Taiye S. Adewumi**	Investigating Host-Viral Interactions in *Drosophila*: Insights from *Drosophila* innubila nudivirus (DiNV)
45	**Tolulope I. Ade**	RpoN-dependent phosphotransferase systems in Enterococcus faecalis
46	**Tristan A. Sprague**	Validation of a Proteoliposome γ-secretase Assay to Investigate Cholesterol's Effect on γ-secretase Function
47	**Vanessa M. Schmidt**	A genetic screen to identify mutations that modulate the effects of quorum sensing on antibiotic resistance in the bacterial pathogen Pseudomonas aeruginosa
48	**Vedant Jain**	A novel model for neuroblast migration and differentiation: Lessons from QL neuroblast migration in Caenorhabditis elegans
49	**Alexa Magstadt**	Small Molecule KRAS Inhibition in Colorectal Cancer
50	**Jacob Kroh**	Using ^{19}F NMR to study the membrane interactions in the N-terminal domain of IpaB

A Special Thanks to Our Sponsors

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. ***CBID: P20GM113117***

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.

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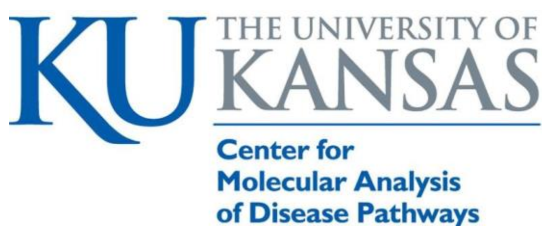
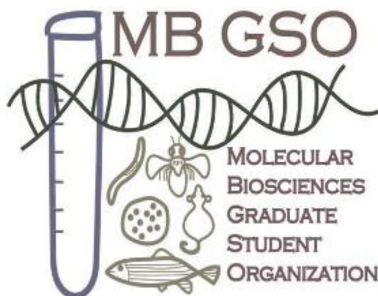


Enabling technologies are critical for the understanding of the biochemical and physical basis of disease as well as its diagnosis. The COBRE CMADP pursues health-related research that is focused on the development and application of these technologies to understanding disease processes. Two of the Center's 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. ***CMADP: P30 GM145499***

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: P20 GM103418



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 Abstract

1. The three-component signal transduction system YesLMN of Enterococcus faecalis senses host glycans to activate expression of an ABC transporter required for host glycan import

Abdulrahman M. Naeem, Janie B. Rainer, Tolulope I. Ade, Zakria H. Abdullahi, Lynn E. Hancock

Enterococcus faecalis, an opportunistic pathogen that normally inhabits the gut in humans has the capacity to utilize a wide range of carbohydrate sources. In E. faecalis, σ54 (RpoN) controls the expression of multiple phosphotransferase systems (PTS) responsible for metabolism of carbon. Through previous work in our lab, it was shown that in the absence of RpoN, alternative carbon sources are required to maintain bacterial growth when grown on glucose as the principal carbon source. Prior transcriptional analysis comparing parental strain V583 with its isogenic rpoN deletion identified the most differentially expressed genes in the rpoN mutant comprising an operon that includes a predicted ABC transporter, EF2223-21 and a three-component signal transduction system (YesLMN). Since YesN is a predicted response regulator, we constructed a yesN mutant and assessed its contribution to the regulation of the operon, as well as potentially other genes regulated by YesN by RNA-seq analysis and confirmed the transcriptomic data by qRT-PCR and luciferase promoter fusions. To assess the contribution of YesL and YesM, a predicted ancillary membrane protein and a membrane bound sensor histidine kinase, we constructed in-frame deletion mutants of both genes and complemented those defects by use of an ectopic integration system to address potential polar effects on YesN regulation and activity. A luciferase reporter transcriptionally fused to ef2223 (the first gene in the operon) allowed us to also address the host glycans that are sensed in a YesLMN-dependent manner and data show that high-mannose type N-linked glycans are sensed by YesLMN.

2. The Autoimmunity-Associated Minor Allele of PTPN22 Enhances Antiviral Immunity During Coronavirus Infection

Alec M. Bevis, Anam Shaikh, Catherine Kerr, Jenna Barnes, Kate Rosa, Tammy Cockerham, Nancy Schwarting, Anthony R. Fehr, Robin C. Orozco

The deadly consequences of viral infection were clearly demonstrated by the COVID-19 pandemic. Therefore, identifying new therapeutic targets to enhance our antiviral immunity and combat virus infection is essential, such as the immune-regulatory gene PTPN22. 5-15% of North Americans express a PTPN22 alternative allele which is linked with multiple autoimmune diseases. However, there is a significant research gap regarding the role of PTPN22 and its common allele during viral infection. To address this, we used CRISPR/Cas9 generated Ptpn22 knockout (PEP-null) and Ptpn22 minor allele expressing (PEP-619WW) mice and infected them with the common murine model of coronavirus, Mouse Hepatitis Virus (MHV) A59. We hypothesize that PEP-null and PEP-619WW mice have enhanced antiviral immunity during coronavirus infection. Following MHV A59 infection, PEP-null and PEP-619WW mice have reduced weight loss and increased survival over WT mice. Additionally, PEP-null and PEP-619WW mice have enhanced innate immunity, such as increased Natural Killer (NK) cell numbers. Furthermore, we show that PEP-null and PEP-619WW innate immune cells enhance protection during MHV A59 infection, but lymphocytes are necessary for survival. These results demonstrate that PEP-null and PEP-619WW are beneficial during coronavirus infection. This research sets the precedent to interrogate the role of Ptpn22 in other RNA virus infections and as a novel therapeutic target to enhance antiviral immunity.

3. EV as a Prognostic Biomarker for Molecular Therapy Targeting RNA-binding Protein HuR in Cancer Immunotherapy

Alfred Buabeng, Sunghae Kim, Qi Zhang, Xiaoqing Wu, and Liang Xu

Breast cancer is highly heterogeneous with diverse molecular alterations. The RNA-binding protein HuR (Human antigen R) plays a crucial role in cancer progression and treatment resistance. Molecular therapies targeting HuR are being developed, but predicting patient response remains challenging. Emerging evidence suggests extracellular vesicles (EVs) are involved in intercellular communication and can serve as biomarkers for cancer diagnosis and prognosis. This study investigates EVs as prognostic biomarkers for therapies targeting HuR in cancer immunotherapy.

EVs from breast and prostate cancer cell lines were isolated and characterized for size distribution and protein markers. HuR expression levels were assessed in cancer cell lines and EVs. In vitro assays evaluated the effect of ISB immunotherapy and HuR small molecule inhibitors on cancer cell viability and EV levels. EVs from cancer cells contained HuR, suggesting HuR transfer between cells via EVs. Treatment with ISB immunotherapy and HuR inhibitors inhibited cancer cell growth, increased T cell activation, and reduced EV levels and surface markers. These findings indicate the potential prognostic value of EVs in HuR-targeted therapy and immunotherapy in breast and prostate cancer.

Further research is needed to understand HuR transfer mechanisms via EVs and validate the in-vivo efficacy of HuR-targeted therapy. This study highlights the potential of

targeting HuR and utilizing EVs in developing personalized cancer treatments.

4. Improving immunity against chronic virus infection using an autoimmunity associated Improving immunity against chronic virus infection using an autoimmunity associated Ptpn22 allele.

Anam F. Shaikh, Alec M. Bevis, Jenna Barnes, Nancy Schwarting, Tammy Cockerham, and Robin C. Orozco

Dysfunctional dendritic cells (DC) lead to inadequate T cell activation causing chronic virus infection. Enhancing both DC and T cell function can prevent chronic virus infection. An approach to enhance immune function is to harness the immune-activating phenotype driven by the autoimmunity-associated allele of PTPN22, which is expressed in all immune cells. We previously found that mice expressing the Ptpn22 autoimmunity-associated allele (Ptpn22-R619W) successfully clear Lymphocytic choriomeningitis virus clone 13 (LCMV-cl13) unlike wildtype (WT) mice. This correlates with a more immunostimulatory DC phenotype and enhanced T cell function. However, the mechanism Ptpn22-R619W enhances DC and T cell function remains unclear. Using our CRISPR/cas9 generated Ptpn22-R619W mice, we addressed the hypothesis that Ptpn22-R619W increases DC immunostimulatory proteins which increases anti-viral T cell activation during virus infection. Post infection, Ptpn22-R619W DCs have reduced infection, increased CD86, and decreased PD-L1 expression both in vivo and ex vivo. Additionally, Ptpn22-R619W DCs have a differential gene expression profile. Also, Ptpn22-R619W mice have enhanced CD8 T cell function against the LCMV-cl13 minor epitope, GP276-280, but not the major epitope. Lastly, co-culture experiments demonstrate that Ptpn22-R619W in both DC and T cells is necessary to enhance T cell function. These results show how Ptpn22-R619W prevents DC and T cell dysfunction during virus infection.

5. Characterization of TolC Colicin Interactions

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

Antibiotic resistance poses a threat to the human health gains made over the past century. In Escherichia coli some antibiotics are removed from the cell by efflux pumps. In Gram negative bacteria, they typically consist of a inner membrane component, a periplasmic component and an outer membrane component. In E. Coli, TolC is a trimeric outer membrane beta barrel, which adapts to multiple efflux pumps allowing small molecules to be removed from the cell. Given this conservation, if we could plug TolC, we could then potentially combat multiple different efflux pumps. Nature has also seen TolCs possibility for cellular access, and evolved proteins which bind into TolC. Colicins are E. coli proteins which use TolC and other outer membrane proteins to deliver cytotoxic payloads to other cells. Colicins, consist of an N-terminal translocation domain (T), a receptor binding domain (R), and a C-terminal cytotoxic domain (C). Several colicins bind TolC via their T-domains to facilitate the translocation of the toxic C-domain into the cell. We already identified that a colicin, Colicin E1 (ColE1), TR is able to reduce efflux mediated resistance. Additionally, we identified three TolC binding colicins, known as ColE1, Colicin 5 (Col5), and Colicin E1* (ColE1*). With our collaborators at Rutgers CryoEM center, along with biophysical characterization we have begun to understand some of the important factors of this interaction. We believe that a continued better understanding this interaction, could yield an effective TolC mediated efflux plug.

6. XPO1 inhibition modulates the Wnt/β-catenin signaling pathway to reduce colorectal cancer tumorigenesis.

Andrew Evans, Dan A. Dixon

Colorectal Cancer (CRC) is the second leading cause of cancer-related death in the U.S. A subset of individuals faces a notably higher likelihood of developing CRC within their lifetime. Hence, there is a compelling need for innovative chemopreventive treatments aimed at minimizing CRC tumorigenesis. Exportin 1 (XPO1; also referred to as CRM1) plays a pivotal role in transporting proteins from the nucleus to the cytoplasm. Numerous cancers overexpress XPO1, including CRC. Selective Inhibitors of Nuclear Export (SINE) compounds, such as Eltanexor, have been developed to target XPO1 and show promising therapeutic benefits. This research evaluates Eltanexor as a chemopreventive agent for CRC. Our findings indicate that in CRC, Eltanexor treatment impairs COX-2 expression by reducing Wnt/β-catenin signaling. Drug sensitivity assays using organoids from Apcmin/+ (a genetic CRC mouse model) tumors and wild-type mouse small intestine tissue show that Apcmin/+ tumoroids are more sensitive to Eltanexor than wild-type organoids. Additionally, when Apcmin/+ mice receive Eltanexor treatment, it significantly reduces tumor burden by approximately 3-fold and decreases the number of tumors larger than 1mm. Collectively, these findings highlight XPO1 as a potent target for CRC chemoprevention.

7. Nuclear APC can regulate 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, roles for APC 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. Here we show that

ApcmNLS/mNLS mice display significantly thinner colonic mucus layers than wildtype mice and also harbor different bacterial species. Additionally, RNAseq data suggest that ApcmNLS/mNLS mice have compromised mucosal barrier integrity and increased retinol metabolism that could result in less goblet cell differentiation. We hypothesize that nuclear APC promotes gut barrier integrity by regulating MUC2 expression. In cultured human colon cells, we showed an upregulation of MUC2 RNA by APC. To extend these findings mechanistically, we showed that APC modulates MUC2 expression through NF-kB inhibition. We have also used chromatin immunoprecipitation (ChIP) to demonstrate an association of APC with MUC2 DNA. 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.

8. PARP14 Restricts HSV-1 Lytic Replication

Anna Ferkul, Hongping Hao, Srivatsan Parthasarathy, Anthony R. Fehr, David Davido

Host defenses dictate the outcome of whether an infection for herpes simplex virus 1 (HSV-1) will be lytic or latent. Poly-ADP ribose polymerases (PARPs) are host proteins that use NAD+ as a substrate to add either poly- or mono-ADP-ribose to substrates (e.g., proteins) and have several functions, including the repair of damaged DNA, regulation of glycolysis, and anti- and pro-viral activities. We and others have observed that 3 PARPs (i.e., PARP1, 9, and 14) are associated with HSV-1 DNA or chromatin. PARP14 is a large, multiple domain protein, that is under positive selection in mammals, indicating an involvement in host-pathogen interactions. Thus, we hypothesized that PARP14 could be a potential restriction factor for HSV-1 replication. Our initial data using two A549 PARP14 knockout cell lines showed that the lack of PARP14 increased the plating efficiency and viral yields of HSV-1 ~50-100-fold compared to control cells. These increases in plating and replication efficiencies correlated with elevated levels (~10-fold) of the immediate-early (IE) transcripts ICP0, ICP4, and ICP27. Use of a PARP14 inhibitor did not alter HSV-1 yields 24 hours post-infection, suggesting that PARP14 impairs HSV-1 lytic replication independent of its ADP-ribosyltransferase activity. Overall, these studies indicate that PARP14 acts as a restriction factor for HSV-1 replication. Future experiments will identify the stage of the viral lifecycle is restricted by PARP14 and the specific domains of PARP14 that contribute to its antiviral activity, as we seek to identify mechanism(s) by which it suppresses viral gene expression.

9. Infectious Disease Assay Development Core: High Throughput Screening Laboratory at the University of Kansas

Anuradha Roy

The overall goal of the IDAD Core is to provide expertise, facilities, services, and training in the area of HTS assay design, development, validation, small and large-scale screening for whole cell based or biochemical infectious disease targets. The IDAD core is an extension of the University of Kansas High Throughput Screening Laboratory which is a fee-for-service, state-of-the-art facility dedicated to providing academia, not-for-profit institutions, biotech, and pharmaceutical industries with exceptional assay development, high throughput screening and data mining services at economical rates. The staff has experience in executing cell-based, biochemical, siRNA as well as high content screening campaigns against a plethora of target classes. The laboratories are equipped with cutting-edge liquid handling and signal detection instrumentation for increasing throughput and precision of screening campaigns. Clients have the option of using our collection of 400,000 compounds and/or a client's own chemical library. KU-IDAD/HTS lab further leverages the strengths of the medicinal chemistry/ computational modeling cores under CoBRE Chemical Biology of Infectious diseases (CBID) program to support your tool/lead discovery research.

10. Role of Haspin kinase in Topoisomerase II Response Checkpoint (TRC) activation in Human Cells

Asbin Chand, Yoshiaki Azuma

Haploid germ cell-specific nuclear protein kinase (Haspin) is a conserved atypical eukaryotic serine-threonine kinase. Haspin phosphorylates Histone 3 threonine 3 (H3T3p); the only known target of Haspin. H3T3p recruits chromosome passenger complex (CPC) consisting of Aurora B kinase. Aurora B maintains genome stability by monitoring spindle assembly checkpoint to ensure correct kinetochore-microtubule (KT-MT) attachment and microtubule tension during early mitosis. DNA topoisomerase II (TopoII) and its unique strand-passage reaction (SPR) in which catenated DNA is resolved are crucial for faithful chromosome segregation. More importantly, this decatenation process is found to be monitored by a TopoII-dependent cell cycle checkpoint called TRC. The TRC is conserved among species. Recent studies have shown that TopoII SUMOylation is key to TRC activation. We have demonstrated that in Xenopus laevis egg extract catalytic inhibition of DNA topoisomerase II by ICRF-193 increases SUMOylation of its CTD. By utilizing SUMO interacting motifs (SIM), SUMOylated TopoII interacts with Haspin which recruits the Aurora B through H3T3p at centromere. However, this molecular circuit needs to be confirmed in human cells.

Moreover, in human cells, Haspin inhibitors completely abolished ICRF-193-induced checkpoint activation but inhibition of TopoII SUMOylation showed partial bypass. However, the exact interactivity of mitotic Haspin and TopoII SUMOylation remains unclear. We will utilize the Auxin Inducible Degron (AID) system combined with the

Tetracycline inducible expression (Tet-ON) system to explore possible mechanisms and interactions between TRC activation and the mitotic behavior of Haspin kinase.

11. Musashi1 regulates mTORC1 pathway, potentially controlling the growth of the intestinal epithelium. **Bikash Pokhrel**, Kristi L. Neufeld

Colorectal cancer (CRC) is the second leading cause of cancer-related death, affecting 1 in every 24 people. CRC develops due to an imbalance in cell division, differentiation, and death in the epithelial cells lining the intestine. Musashi1, an RNA-binding protein, is exclusively expressed in stem and dividing cells and is essential for their maintenance and fate. Our published research showed that ubiquitous overexpression of Musashi1 in mice resulted in reduced growth of the organism and various organs. We employed bulk RNA sequencing and in-vitro studies to determine the mechanism underlying this growth defect. Our studies revealed that Musashi1 inhibits growth by regulating the mTORC1 pathway. The mTORC1 pathway is central to cellular growth, as it controls both anabolic processes like protein and lipid synthesis and catabolic processes like autophagy. Immunofluorescence studies revealed that Musashi1 protein and mTORC1 activity are mutually exclusive in cells of the intestine, consistent with a key role for this interaction in determining the fate of stem and dividing cells. Our current work aims to identify the RNA targets of Musashi1 that regulate the mTORC1 pathway. Given that mTORC1 and Musashi1 are often dysregulated in CRC, our study may lead to improved treatment strategies.

12. Characterizing C. acnes and Staphylococcus spp. in polymicrobial biofilms
Bren Den Ng, Danielle S. Williams, Rosana B.R. Ferreira

Polymicrobial biofilms are frequent causes of chronic infections and a serious medical problem due to antimicrobial resistance. Staphylococcus spp. and Cutibacterium acnes are common skin commensals and are usually beneficial, but can cause chronic infections of prosthetic- devices and are frequently co-isolated from these infections. Our group has shown that S. hominis and S. lugdunensis produce substantially more biofilm when co-cultured with C. acnes compared to monocultures. This phenotype is specific to this combination of species. In this project, we aim to better characterize the interactions among these three species, focusing on identifying the mechanism by which the interaction with C. acnes causes increased biofilm formation in S. lugdunensis and S. hominis. For that, we inoculated S. lugdunensis and C. acnes in monoculture or in co-culture using different concentrations of each species and incubated them at 37 oC for 72h under anaerobic conditions and assay for biofilm production. We found that concentrations as low as 1% of S. lugdunensis can still display the same strong biofilm formation in the presence of C. acnes. However, at least 10% of C. acnes is needed for the phenotype to be observed in the presence of S. lugdunensis, suggesting a major role for C. acnes on this interaction. We now plan to quantify each species in polymicrobial biofilms over time and evaluate the impact of co-culture on global gene expression of each species. The results and subsequent characterization of the interaction will allow us to combat the polymicrobial infection by targeting the mechanisms involved.

13. LasR and FusA1-regulated ceftazidime resistance is dependent on stringent response in Pseudomonas aeruginosa
Brielle M. McKee, Rhea Abisado-Duque, Kade Townsend, Kate Woods, Josephine R. Chandler

Pseudomonas aeruginosa is a common multi-drug resistance human pathogen that can cause long-term and difficult to treat infections. Throughout infection, adaptive mutations arise and can alter resistance to clinically relevant antibiotics, such as tobramycin and ceftazidime. Common mutations occur in the genes lasR and fusA1, which respectively, encode for a quorum sensing regulator and a translation accessory protein. Despite the abundant prevalence of lasR and fusA1 mutations in chronic infections and their known role in modulating resistance to tobramycin, research regarding their impacts to ceftazidime resistance is limited. In prior work, we showed mutations in lasR and FusA1 (A21T) significantly decrease resistance to ceftazidime, indicating a previously unknown role for LasR and FusA1 in ceftazidime resistance. To expand on the role of FusA1, we tested the MIC of several FusA1 mutants and discovered that they yield a large range of effects on ceftazidime resistance and are largely dependent on LasR. As observed, FusA1 L40Q increases resistance ~5 fold compared to the PA14 wildtype and ~100-fold compared to a lasR-null mutant. However, the effects of FusA1 L40Q appear to only be significant during stationary phase when stress regulatory networks like quorum sensing and stringent response are most active. Because FusA1 and LasR have both been previously linked to the regulation of stringent response, we hypothesized that stringent response is important for LasR and FusA1- mediated ceftazidime resistance. In support of this hypothesis, we observed that deleting stringent response from PA14 and FusA1 L40Q abolishes ceftazidime resistance. There is also preliminary evidence to suggest that stringent response is downregulated in the FusA1 A21T mutant, which could explain its decrease in ceftazidime resistance. Current efforts are underway to better understand the regulatory effects on stringent response by LasR and FusA1 and the mechanism by which they alter ceftazidime resistance. Overall, this research furthers our current understanding of the evolution of antibiotic resistance and points to potential novel mechanisms of ceftazidime resistance in P. aeruginosa.

14. The Synthetic Chemical Biology Core (SCB): A Resource for Research in Chemical Biology
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 synthesis 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.

15. The core programmed cell death pathway regulates neuronal migration downstream of the MAB-5/Hox transcription factor in C. elegans

CJ Gormly, Erik A. Lundquist

Neuroblasts QL and QR are born bilaterally in the posterior region of C. elegans between the V4 and V5 hypodermal seam cells. QR migrates anteriorly over V4 and QR migrates posteriorly over V5, and both cells divide. EGL-20/Wnt is secreted by muscle cells near the anus where QL daughter cells QL.a/p encounter it. EGL-20 drives the expression of MAB-5 in QL via canonical Wnt signaling. MAB-5 is both necessary and sufficient to drive posterior migration. Before MAB-5 re-programs QL.a to migrate posteriorly, it also prevents anterior migration of QL./p, which is a default state. FACS sorting and RNA-seq was performed in early L1 Q cells from mab-5 gain-of-function (gof) and wild-type worms to identify genes acting downstream of MAB-5. Differential expression analysis identified genes that were upregulated in the mutants compared to the wildtype. egl-1 was significantly upregulated in the mab-5 gof. Canonically, EGL-1 activates the programmed cell death pathway by inhibiting CED-9/Bcl2, however the PCD pathway is involved in other developmental events such as synapse elimination (Meng et al., 2015). egl-1 mutations had no effect on posterior QL migration alone, but significantly enhanced anterior QL.ap (PQR) migration defects of a hypomorphic egl-20 mutant. ced-4/APAF also strongly enhanced anterior PQR migration in egl-20 and bar-1 hypomorphic mutants. These results suggest that EGL-1 and CED-4 are required to inhibit the anterior migration of QL.a and QL.p. Future studies are aimed at characterizing other PCD pathway members in this process, and determining the mechanisms by which this pathway might inhibit anterior migration.

16. Mechanisms of genome scaling during embryogenesis and evolution

Coral Y. Zhou, Bastiaan Dekker, Ziyuan Liu, Hilda Cabrera, Joel Ryan, Job Dekker, Rebecca Heald

Across the tree of life, genome size scales with nuclear size and cell size, yet underlying mechanisms are largely unknown. Using a combination of in vitro and in vivo approaches unique to the African clawed frog Xenopus laevis, we recently discovered the molecular mechanisms underlying how mitotic chromosomes scale in size during the rapid and reductive cell divisions in the early embryo. We are currently expanding our toolkit to study mechanisms of genome scaling across multiple spatial scales of 3D genome organization and function, and in the context of early development, evolution and disease.

17. 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.

18. Determining a roll for Nuclear APC in UV-Induced DNA Damage Repair

Eldric Carreon, Kristi Neufeld

Colorectal cancer (CRC) is the second most deadly cancer in the US, with an estimated 152,810 new diagnoses and 53,010 deaths in 2024. Mutation of Adenomatous polyposis coli (APC) occurs in ~80% of CRCs. We previously showed that APC, a known cytoplasmic regulator of Wnt signaling, is capable of nucleocytoplasmic shuttling and identified nuclear roles for APC in Wnt signaling as well

as cell cycle control. Other proposed nuclear APC functions include 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. Based on these findings, we hypothesize that APC has a crucial role in UV-induced DNA damage response.

To more directly examine if APC participates in DNA damage response, we obtained a human colon cancer cell line null for APC and used CRISPR to re-introduce wild-type APC into these cells. Six individual clones were characterized for cell cycle/growth characteristics and response to UV exposure. Cells with or without APC (APC+ and APC-, respectively) were exposed to 10 J/M2 UV and monitored at 50 minutes and 24 hours post-exposure. DNA damage was assessed by staining cells for phosphorylated H2AX (□H2AX), a known marker for double-strand DNA breaks, but also involved in other types of DNA damage response. Our results showed no difference in response at 50 minutes post-UV. However, 24 hours post-UV, APC+ cells had recovered more fully compared to APC- cells. Given APC’s proposed role in cell cycle regulation, we explored whether the altered response to UV could result from this feature. We found that APC+ and APC- cells displayed similar growth and cell-cycle characteristics prior to UV exposure. In contrast, 24 hours post UV exposure, a significantly higher percentage of APC- cells were in the sub-G1 and S-phases compared to APC+ cells. These results are consistent with a role for APC in the repair of DNA damage resulting from UV exposure, with the possibility that cells lacking APC accumulate stalled replication forks and remain in ‘S’ phase or else undergo cell death. Current experiments are aimed to determine whether stalled replication forks differ between APC+ and APC- cells and elucidate the precise role of APC in this process.

19. PARP14 in Host-Virus Interactions: Identifying Domains Required for IFN-I Induction and Viral Replication Control

Srivatsan Parthasarathy, **Pradtahna Saenjamsai**, Hongping Hao, Anna Ferkul, Jessica J. Pfannenstiel, Daniel S. Bejan, Yating Chen, Nancy Schwarting, Masanori Aikawa, Elke Muhlberger, Robin C. Orozco, Christopher S. Sullivan, Michael S. Cohen, David J. Davido, Adam J. Hume, Anthony R. Fehr

PARP14, a 203 kDa multi-domain protein with ADP-ribosyltransferase activity, plays diverse roles in cellular functions and is implicated in antiviral responses. Upregulated during viral infections and evolved under positive selection, PARP14's specific role in host-pathogen interactions remains unclear. Our research investigates PARP14's direct antiviral functions and its impact on interferon (IFN) production and viral replication.

PARP14 enhances type I and III IFN responses and restricts replication of multiple viruses, including ADP-ribosylhydrolase (ARH)-deficient murine hepatitis virus (MHV) and Herpes simplex virus 1 (HSV-1). Conversely, PARP14 is critical for efficient replication of vesicular stomatitis virus (VSV), Ebola virus (EBOV), and Nipah virus (NiV). Notably, a PARP14 catalytic inhibitor did not affect HSV-1 or EBOV infection, suggesting multiple domains impact virus replication.

To elucidate the mechanisms of PARP14's antiviral immune functions, we aim to identify which domain(s) promote IFN-I induction following poly(I:C) treatment and which domain(s) impact viral replication. We generated six N-terminal and five C-terminal PARP14 truncation mutants and integrated them into A549 PARP14-knockout cells using lentiviral vectors. By stimulating these cells with poly(I:C) and comparing IFN-I responses to full-length PARP14 and complete deletion. Additionally, we will infect cells with different viruses and compare viral replication to full-length and complete deletion mutants. We aim to determine the structural requirements for PARP14's role in IFN-I production and viral replication.

This ongoing research provides new insights into PARP14's complex role in host-pathogen interactions, potentially leading to novel targets for antiviral therapy.

20. Transcription patterns of quorum-sensing regulated genes in Chromobacterium subtsugae differ under induction by native and competitor signals.

Dr. Josephine Chandler, **Eryk Yarkosky,** Samalee Banarjee, Cristina Vargas

Quorum sensing is common to many bacteria and can have broad effects on susceptibility to antibiotics, pathogenesis, and competition with other strains and species. In Chromobacterium subtsugae, there is a single quorum-sensing system that produces and responds to the signal molecule N-hexanoyl-L-homoserine lactone (C6-HSL). Upon binding to C6-HSL, the receptor CviR changes conformation such that it can activate a set of target genes through recognizing a semi-conserved site within the target gene promoter called a “lux box”. Previous studies show that CviR can sense and respond to other signals in addition to C6-HSL, and that this broad signal selectivity affords C. subtsugae a competitive advantage, which we call “eavesdropping.” Here, we ask whether different signals can elicit activation of different promoters by CviR. We used RNA-seq to show that C. subtsugae activates distinct regulons in response to two different signals; the native signal C6-HSL and a non-native signal, C8-HSL. I also used gene promoter fusions to the reporter lacZ to determine the sensitivity of several CviR-regulated gene promoters to C6-HSL and C8-HSL by generating dose-response curves. Initial reporter results in C. subtsugae support the idea that different gene promoters have distinct responses to these two signals. Next, I will examine the lux-box like sequence of the CviR-activated promoters and make mutations to CviR, altering signal selectivity to determine the relative contributions of DNA sequence and CviR-ligand

interactions to this differential signal response. The results are expected to shed new light on how quorum sensing allows bacteria to eavesdrop on their competitors and the mechanisms involved in such responses.

21. RNA-RNA Interaction of a non-coding region in Hepatitis C Virus Genome Promotes Translation

Ethan Rogers, Erik Holmstrom, Parker Sperstad

Hepatitis C is a liver inflammatory disease caused by the Hepatitis C Virus (HCV), chronic infections of which can result in liver cirrhosis or cancer. The HCV genome is a single-stranded, positive-sensed RNA molecule with three general domains; The 5' untranslated region (5'UTR) that contains an Internal Ribosome Entry Site (IRES), an open-reading frame that encodes a 10-protein polyprotein, and a 3' untranslated region (3'UTR). The viral genome is highly structured with specific RNA-RNA interactions that are critical for synthesis of viral protein or (-) RNA. One such RNA-RNA interaction is between the 3'X, found at the end of the 3' UTR, and 5BSL3.2, found within the open-reading frame. 3'X has been demonstrated to be an essential element of viral replication that is involved in various replicative processes. The 3'X has also been shown to adopt two coexisting conformations that can slowly interconvert. The equilibrium of these two conformations is dependent on [Mg2+(aq)]. 5BSL3.2 has been shown to be a key regulator of protein synthesis by binding to the IRES, which inhibits viral protein synthesis. This study explored how the binding affinity of 3'X-5BSL3.2 is a function of [Mg2+(aq)]. Our results show that as [Mg2+(aq)] was titrated, the binding affinity of 3'X increases for 5BSL3.2. Based on these results, we believe that this interaction would leave the IRES free for ribosome binding, promoting viral protein synthesis.

22. Mitotic activities of Ewing sarcoma proteins

Evan Schulz, Mizuki Azuma

The hallmark of Ewing sarcoma (ES) is the t(11:22) chromosomal translocation, generating two aberrations of the EWS protein, denoted as EWS aberrations: the loss of an EWS allele and expression of EWS/FLI1 fusion. We established an engineered DLD-1 colorectal cancer line with inducible EWS/FLI1 expression using doxycycline (Tet-On system), and single-allele EWS knockdown using auxin (auxin-inducible degron system). Immunocytochemistry revealed that the cells treated with auxin and/or doxycycline for seven days displayed higher EWS signal intensity in interphase and mitosis. The cells with high EWS intensity also showed a positive correlation between EWS/FLI1 signal intensity. Since EWS regulates mitotic spindle acetylation, we investigated the levels of tubulin acetylation in these samples and observed EWS aberrations increased acetylated tubulin signal intensity of mitotic apparatuses. Likewise, interphase and mitotic ESs show higher acetylated tubulin signal intensity than MSCs. Based on this data, we hypothesize that the cells with EWS aberrations undergo a selection process to overexpress the EWS during ES development. Supporting our hypothesis, western blotting suggested that EWS expression is higher in ES than mesenchymal stem cells (MSCs), the cell of origin of ES. Bioinformatic analysis of the NCBI RNA-seq database also shows the upregulation of EWS in ES cells when it is compared to other sarcomas and normal bone. Currently, we are investigating the molecular mechanisms of EWS upregulation and its effect on tumorigenesis.

23. Transcriptomic differentiation map of a C. elegans neuroblast lineage at single-cell resolution

Felipe L. Teixeira, Erik A. Lundquist

Cell migration is a crucial mechanism for proper development of the nervous system, and the asymmetrical migration of Caenorhabditis elegans Q neuroblasts serves as a useful model to study the importance of such mechanism for neuronal development. C. elegans Q cells are bilateral neuroblasts born at similar positions on the left (QL) and right (QR) lateral side of the animal. Despite the position similarity, QL and QR will follow an asymmetrical pattern of migration. During the first hours after hatching, QL will migrate posteriorly to a position dorsal to the seam cell V5 and QR will migrate anteriorly to a position above V4. After reaching these positions, the Q cells will divide and their descendants migrate further along the anterior-posterior axis. To better understand this asymmetric migration, we are using single-cell RNA-sequencing (scRNA-seq) to study changes in gene expression associated with migration and differentiation of the Q neuroblast lineage. Q cells containing two markers that are co-expressed exclusively on them (Pegl-17::gfp; Pscm::mCherry) were isolated by FACS and used for scRNA-seq analysis. Sequenced cells represent different stages of the Q lineage development, so different known marker genes were used to identify the different cells in each cluster. Thus far, our scRNA-seq data depict a robust transcriptomic differentiation map of the Q neuroblast lineage, which has shown that the similarity between left- and right-side Q neuroblasts during development appears to be greater than expected, demonstrating the developmental robustness of the Q cell lineage despite the asymmetry in migration.

24. Bioactive small molecules from the human gut microbiome modulate Salmonella enterica invasion gene expression

Chukwuma Great Udensi, Caetano Antunes

The human gut harbors a vast community of microorganisms known as the gut microbiome, which plays a crucial role in human health by preventing pathogen invasion. Gut commensals achieve this by competing for nutrients, attachment sites, and other unknown mechanisms. Here, we demonstrate that ethyl acetate extracts from pure cultures of Enterocloster citroniae and Enterocloster aldenensis, human gut commensals, suppress the expression of hilA, a key regulator of Salmonella host cell invasion. We previously showed that aromatic compounds from the

human gut metabolome, including 3,4-dimethylbenzoic acid (DMB), strongly repress hilA expression. Here, we screened aromatic compounds from the Infectious Disease Assay Development Core library for compounds structurally related to DMB and identified other small aromatics with anti-invasion activity. Furthermore, to identify the chemical cues produced by E. citroniae responsible for the inhibitory activity against Salmonella invasion gene expression, bioactive compounds were separated using Reverse Phase High-Performance Liquid Chromatography on a C18 column. Surprisingly, no fractions showed activity, suggesting that a compound mixture is required for activity. Nevertheless, we will continue our HPLC efforts using different chromatographic methods. Once active fractions are obtained, mass spectrometry and nuclear magnetic resonance spectroscopy will be performed to identify bioactive compounds. The effect of pure bioactive compounds on Salmonella gene expression will be monitored using quantitative real-time PCR targeting invasion and other virulence genes. Together, these results will enhance our understanding of host-pathogen interactions and could lead to the development of novel small-molecule inhibitors against enteric pathogens in humans and animals.

25. Dalton Lab Overview: Rare Diseases, Drug Screens, and Essential Genes

Hans M. Dalton

There are hundreds of genes absolutely necessary for life. Hypomorphic mutations in these genes lead to rare, debilitating diseases. Paradoxically, impairing multiple essential genes can sometimes be better than a single mutation, and loss of essential genes later in life can instead sometimes be beneficial. The Dalton lab studies these genes in multiple ways: 1. creating disease models in human cells or fruit flies and performing drug screens to find new therapeutics; 2. examining their genomic origin, e.g. how conserved are they, and are there duplication events? and 3. understanding their role throughout life history, i.e. development vs. aging. Our lab intersects human disease and basic biology through these genetic pathways. This poster will provide an overview of the Dalton lab and give an idea of the projects we will work on. Dr. Dalton is a new professor at KU and would be happy to meet you!

26. Role of EWSR1 allele loss and EWSR1-FLI1 fusion in aneuploidy induction of Ewing sarcoma

Harsha Hapugaswatta, Mizuki Azuma

Ewing sarcoma is a pediatric bone cancer characterized by a chromosomal translocation between chromosome t(11;22) causing one allele loss of *EWSR1* and gaining *EWSR1-FLI1* fusion. To study this DNA aberration, we established a cell line that enables EWSR1 knockdown with Auxin (AUX) treatment, and EWS-FLI1 expression using a Tet-on system with Doxycycline (DOX) treatment. None of the drug-treatment groups (AUX+, DOX+, and AUX+/DOX+) showed any change in cell viability compared to the control (DMSO) cells. However, increased cell size was observed in all three sample groups compared to the control cells. Specifically, the percentage of smaller cells (200-500 μm^2) was significantly ($P<0.05$) reduced, whereas the percentages of larger cells (501-1000 mm^2, 1001-2000 μm^2) were significantly ($P<0.05$) increased compared to control cells after 2-8 days of treatments. Fluorescence-activated Cell Sorting analysis (FACS) revealed that there was a twice higher percentage of larger cells in the three sample groups compared to control cells. Furthermore, we analyzed the DNA content of large and small cells using Hoechst staining. Small cells displayed normal ploidy (2N and 4N), however, the majority of large cells carried aberrant numbers of chromosomes (>4N). Despite Ewing sarcoma cell morphology is known as a small round cell, we observed cell size variation in A673 Ewing sarcoma cells. Our data suggests that one allele loss of *EWSR1* and *EWSR1-FLI1* fusion induces aneuploidy and increases cell size.

27. 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 NextSeq2000 and NextSeq550 (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/.

28. Mutations affecting different biochemical functions of the coronavirus macrodomain, Mac1, indicate that it promotes multiple stages of the viral replication cycle

Joseph J. O'Connor, Anu Roy, Yousef M. Alhammad, Reem Khattabi, Philip Gao, Anthony R. Fehr

All coronaviruses (CoVs) encode a conserved macrodomain, termed Mac1, in non-structural protein 3 (nsp3) which binds and hydrolyzes ADP-ribose covalently attached to proteins. Mac1 is a key virulence factor that counters antiviral ADP-ribosylatransferase (PARP) activity. Previously, we found that MHV with a mutation in the adenine binding site, MHV-D1329A, was extremely attenuated in all cell culture models of infection, as opposed to MHV-N1347A, which only has a replication defect in bone-marrow derived macrophages (BMDMs). Interestingly, an N1347A/D1329A double mutant was unrecoverable, indicating an essential role for Mac1 in infection. Based on this prior work, we hypothesized that these mutations may impact different stages of the viral lifecycle. First, to clarify the impact these mutations have on the biochemical activities of Mac1, we generated recombinant SARS-CoV-2 Mac1 proteins encoding the same mutations. As expected, the D-A mutation was extremely defective in ADP-ribose binding, but maintained some enzyme activity, while the N-A mutation had nearly WT levels of ADP-ribose binding but had low enzyme activity, confirming that these mutations have different effects on the biochemical functions of Mac1. Next, we analyzed how MHV D1329A and N1347A viruses impact the viral lifecycle. In BMDMs, N1347A infected produced normal levels of viral genomic and sub-genomic RNA, but had reduced levels of viral protein, indicating a defect in protein translation. In contrast D1329A produced viral non-structural protein 3 early during infection, but displayed a large defect in the accumulation of viral genomic and sub-genomic RNA compared to WT or N1347A. These results suggest that Mac1 binding and enzymatic activities are critical for different stages of the viral lifecycle, demonstrating the critical importance of Mac1 for MHV replication.

29. A *Drosophila* DNA Virus Model

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

Drosophila innubila Nudivirus (DiNV) is a DNA virus that infects the mushroom feeding fly *Drosophila* innubila (Dinn). In our lab, with the goal of developing a DNA virus model in *Drosophila*, we have explored the specificity of DiNV infection in insect cell lines derived from *Drosophila* innubila (Dinn), D. virilis (Dv-1), D. melanogaster (S2), Spodoptera frugiperda (Sf9) and Heteronychus arator (HA1179). Our results indicate DiNV grows in Dinn and Dv-1 cells, moderately in S2 cells and not at all in Sf9 and HA1179 cells. DiNV derived directly from wild caught flies can infect the cells and replicate but fluids from these cultures appear to not contain progeny virions that can reinfect new cells. In contrast, a cell culture adapted DiNV demonstrated similar infectibility in the three *Drosophila* cell lines and fluids derived from these infected cells can reinfect new cells. The determination of DiNV infection specificity in cell culture allows for detailed molecular studies of DiNV interactions in cell culture and the host animal.

30. Differential Evolution of Pseudomonas fluorescens against Ribosome-Targeting Peptide vs Small Molecule Antimicrobials

Kervens Accilien, Robert Unckless

Antibiotics are a powerful tool used to reduce the impact of pathogen infections on human health and quality of life. Traditional single-molecule antibiotics function by binding to and inhibiting crucial bacterial machinery, such as polymerase, topoisomerase, and ribosome, ultimately leading to bacterial cell death and population collapse. However, bacteria can sometimes evolve and become resistant to the antibiotics they are exposed to. The development of new antibiotics does little to solve this problem, as resistance often follows shortly after exposure. One potential solution to this issue is the development of new classes of antibiotics that are harder for bacteria to develop resistance against. Early studies suggest that bacteria are less likely to develop resistance against peptide antibiotics. To further explore this, we will closely examine the differences between bacteria resistant to peptides and those resistant to single-molecule antibiotics. To do this, we will select a group of antimicrobial peptides and single-molecule antibiotics that target the same biological function (peptide synthesis). We will determine the minimum inhibitory concentration of each antibiotic type and then generate antimicrobial-resistant populations through experimental evolution. These populations will then be sequenced and compared to the original population to identify the mutations that cause resistance. By comparing the mutations in resistant bacteria from the two different antibiotic classes, we hope to gain a better understanding of how bacteria adapt to these antibiotics and how we can use this knowledge to prevent resistance development.

31. Staphylococcus epidermidis-diffusible molecules inhibit Staphylococcus aureus virulence

Kiana Hajiarbabi, Rayssa D. Lima, Nathalia S. Magalhães, Rosana B. R. Ferreira

The human microbiota plays a crucial role in health and disease, with much of the research focusing on the gut. However, the role of the skin microbiota as a primary defense against pathogens remains less understood. This study investigates the activity of molecules produced by a commensal S. epidermidis isolate against Staphylococcus aureus biofilm formation and its adhesion and invasion of host epithelial cells. Previous findings revealed that molecules present in the cell-free culture medium (CFCM) of S. epidermidis strain RF1 significantly reduce S. aureus biofilm formation. In this project, we evaluated the impact of these molecules on S. aureus adherence and invasion of human epithelial host cells. Our results showed that growth of S. aureus (1602 and JE2) in the presence of S. epidermidis

CFCM significantly reduced both the adhesion and invasion of S. aureus in A549 cells. Cell viability and toxicity were determined using LDH and MTT assays and, showed that S. epidermidis CFCM at 5% or 1% concentrations did not significantly impact A549 cells. Biofilm assays demonstrated that S. epidermidis CFCM at both concentrations significantly reduces biofilm formation of S. aureus. To identify the bioactive molecules responsible for these effects, we performed liquid-liquid extraction using organic solvents, finding that the bioactive molecules were soluble in butanol and ethyl acetate. Further analysis will be performed to identify these molecules. These findings highlight the potential of S. epidermidis-derived molecules in combating S. aureus infections and underscore the importance of further exploring the skin microbiota for therapeutic applications.

32. The Impact of PTPN22 on the B Cell Landscape During Viral Infection

Proctor-Roser, Macie, Orozco, Robin

Presenting Author Persistent viral infections affect millions of people globally. B cells play a crucial role in viral clearance and orchestrating immune memory during persistent infections. They assist CD4+ T cells as antigen presenting cells and are the exclusive producers of antibodies. Protein Tyrosine Phosphatase Non-Receptor Type 22 (PTPN22), is expressed in all immune cells, encoding for PEP (mice)/LYP (humans). The lack of PTPN22 establishes a selection bias promoting Follicular over Marginal Zone B cells, potentially changing the overall B cell landscape during viral infection. Further, mice lacking PTPN22 (PEP-Null) can clear persistent Lymphocytic choriomeningitis virus clone 13 (LCMV-Cl13) infection, whereas PEP-WT cannot. However, the role of PTPN22 in B cells during virus infection is unknown. To assess this, we quantified B cell subsets in PEP-WT and PEP-Null mice during LCMV-Cl13 infection. In naïve animals, PEP-Null mice have more Follicular, Marginal Zone, and Plasmablast B cells compared to PEP-WT mice. Following infection PEP-Null mice have less Follicular B cells, but more Plasmablasts compared to PEP-WT. This suggests that PTPN22 could be impacting quick short-term Plasmablast antibody production, and PEP-Null mice are producing antibodies at an earlier time point than that of a typical adaptive immune response. Clearance of LCMV-Cl13 in PEP-Null mice could be due to these fast-acting antibodies. Understanding these mechanisms would advance our understanding of the critical role B cells play in persistent infections and could lead to potential avenues for vaccine development.

33. Inhibitors of CDK-1 and CDK-2 Diminish HSV-1 Immediate-Early Gene Expression

Maxim Rodzkin, Drew Honeycutt, David Davido

Herpes simplex virus 1 (HSV-1) is a DNA-enveloped virus causing oral, facial, or genital sores and, in severe cases, keratitis and encephalitis. As an obligate intracellular pathogen, HSV-1 exploits cellular factors, including host transcription machinery. Cyclin-dependent kinases (CDKs) are crucial regulators of transcription, interacting with components of the transcription machinery and modifying chromatin structure. Evidence suggests that cell cycle-related CDKs, such as CDK-1 and CDK-2, may be involved in both host and viral gene transcription. Previous studies from our lab have shown that the broad-spectrum CDK inhibitor Roscovitine reduces HSV-1 immediate-early (IE) transcript levels. To determine which specific CDKs modulate HSV-1 replication and gene expression, we performed viral yield assays using inhibitors of CDK-1, CDK-2, and CDKs-4/6, observing that inhibitors of CDK-1 and CDK-2 significantly impaired wild-type virus replication. Subsequent reverse transcription-qPCR and western blot experiments showed that CDK-1 or CDK-2 inhibition led to a substantial reduction in IE gene expression as early as 3 hours post-infection (hpi). To identify CDK-1 targets involved in IE transcription, we performed high-throughput tandem mass tag (TMT) mass spectrometry to examine changes in the host and viral phosphoproteomes early during infection upon CDK-1 inhibition. These phosphoproteomic analyses suggest that the large subunit of RNA polymerase II is one of several host factors potentially phosphorylated by CDK-1. We propose that CDK-1 phosphorylates RNA polymerase II to stimulate viral IE transcription. Future experiments will examine the roles of CDK-1 and CDK-2 and their effects on specific targets in the regulation of HSV-1 transcription.

34. HuR Regulates the cGAS-STING Pathway: A Potential Therapeutic Target in Cancer Immunotherapy

Ngoc Huan Nguyen, Sunghae Kim, Xiaoqing Wu, and Liang Xu

Cancer immunotherapies have achieved significant success, but many challenges still remain. Therefore, discovering new approaches is crucial. The cGAS-STING pathway is a cytosolic DNA sensor that activates the innate immune system in response to infections, inflammation, and cancers. Human antigen R (HuR), also known as HuA or ELAVL1, is an RNA-binding protein that has been studied as a potential therapeutic target in cancer treatment. HuR is involved in various post-transcriptional regulatory processes, including mRNA splicing, maturation, nuclear export, stability, and translation. Consequently, HuR dysfunction contributes to various diseases, including cancer. In our laboratory, we have developed KH3 and modified compounds that can inhibit the interaction between HuR and its mRNA targets. Growth curve comparison experiments have demonstrated that HuR knock-out (KO) cells grow much slower than the parental PC3 cells. Additionally, MTT assays have indicated that KH compounds reduce the survival rate of PC3 cells. Western blot analysis has revealed differential protein expression levels in the STING pathway, both in HuR knock-out cells

and cells treated with KH compounds. These findings suggest that HuR may play a role in regulation of cGAS-STING pathway, and HuR inhibition leads to activating immune responses such as the production of type I IFNs and chemokines, which in turn induce the infiltration and activation of cytotoxic T cells and natural killer cells at tumor sites. This discovery offers a promising strategy for cancer immunotherapy.

35. Insights into the Isoenergetic Monomeric Structures of the Hepatitis C Virus 3'X RNA

Sperstad, Parker, Holmstrom, Erik D

The hepatitis C virus 3′X RNA is a highly conserved, 98-nt sequence at the 3′ terminus of the genome with two isoenergetic conformations. These two conformations differ in the base paring in the first 55-nt and are notably distinguished by the organization of a palindromic 16-nt sequence called the dimer linkage sequence (DLS) that is responsible for genome dimerization. One conformation, DLSa, is a single stem-loop and presents the DLS in the apical loop. Conversely, the second conformation, DLSb, is a two stem-loop conformation that buries the dimer initiating bases in a stem. Here, we monitored the conformational status of monomeric 3′X RNA using single-molecule FRET to further understand which conformation is formed at any given time or under a given set of conditions and how they might interconvert. We have observed the two monomeric conformations with their relative abundances dictated by either solution conditions or nucleotide deletions.

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

Peter R. McDonald, Robin C. Orozco, 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, an Agilent NovoCyte Advanteon conventional flow cytometer, and other supplemental assay instrumentation. The flow cytometry analyzers provide users with tube- and plate-based, conventional and spectral flow cytometry. 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 is equipped to handle BSL-2 samples and perform aseptic and single cell sorting into tubes or 96-well plates. The facility provides instrument training for users who desire to become self-operators of the facility instruments. The FCC will equip CBID researchers with tools directly applicable to infectious disease research, such as identifying and characterizing infectious agents such as bacteria and parasites, quantification and sorting of cells infected with microbial pathogens, and assessing chemical probe efficacy against infectious agents. The University of Kansas Flow Cytometry Core seeks to assist the academic community in achieving their research goals.

37. University of Kansas Biomedical Research Core Facilities

Peter R. McDonald, Erik A. Lundquist

Core labs at KU benefit university investigators and industry partners alike. Each lab has experienced professionals and state-of-the-art technology to support you throughout your project. Working with core research labs provides multiple benefits: (1) full-time technicians to train you to use selected equipment, (2) temporary expertise for your project when you need it, (3) expert consulting on research design and proposal development, and (4) consultation on manuscript preparation. For external partners, outsourcing research to KU's state-of-art facilities has helped our corporate partners defray costs, extend capabilities, access expert scientific advice and service, and solve problems. If you are interested in working with one of our labs, contact the director to discuss capabilities, equipment availability, service rates and policies. There is no contract necessary for academic researchers, but if you should require a nondisclosure agreement to discuss a project, contact KU's Agreements Group at indcontracts@ku.edu or 785-864-7403. The University of Kansas is committed to advancing research and academic endeavors at both KU and neighboring scientific communities. Our research core facilities offer a wide variety of services, ranging from cell sorting to electron microscopy to X-ray crystallography. Through these diverse resources, we provide access to state-of-the-art equipment and instrumentation, technical expertise, training and education all designed to support innovative, cutting-edge research.

38. Cutibacterium acnes Secretes Molecules that inhibit Staphylococcus lugdunensis biofilm formation, adhesion and invasion to human epithelial cells

Rayssa Durães Lima,Rosana Barreto Rocha Ferreira

Staphylococcus species are frequently isolated from human and animal infections, both in hospitals and in the community. The rapid spread of antimicrobial resistance among these species indicates the importance of discovering new alternatives for the treatment of such infections. Biofilm formation is an important way in which these species establish and persist in the host and the environment. Infections associated with biofilms are more difficult to treat, highlighting the importance of compounds capable of

acting on these structures. Cutibacterium acnes, an important member of the human skin microbiome was shown to protect the skin against certain pathogens. In previous work, we investigated whether molecules produced by C. acnes could affect the biofilm formation of Staphylococcus species. We have seen that these molecules have antibiofilm activity against S. lugdunensis without affecting its planktonic growth. In the present study, we demonstrated that these molecules affect the initial steps of S. lugdunensis biofilm formation. Afterwards, we assayed for the ability of these molecules to inhibit adhesion and invasion of S. lugdunensis in epithelial cells, and we observed that they could inhibited S. lugdunensis adhesion and invasion. S. lugdunensis gene expression was evaluated in the presence of C. acnes molecules and 174 genes were differentially expressed. Finally, the cell-free conditioned medium obtained from C. acnes was fractionated using the High Performance Liquid Chromatography (HPLC) and a fraction with significant antibiofilm activity was obtained. Understanding the interactions between different microorganisms could shed light on new compounds with potential applications to help treat and prevent bacterial infections.

39. Determining the Functions of Topoisomerase II as an Enzyme and as a Structural Component on Mitotic Chromosomes

Regan Krueger

DNA Topoisomerase II (TopoII) is an enzyme used for detangling entwined genomic DNA. Additionally, TopoII is identified as a main component of the chromosomal scaffold which is proposed to aid in the structural organization of mitotic chromosomes. At the cellular level, these functions of TopoII in mitosis have been shown to require its C-terminal domain (CTD), which is dispensable for DNA detangling activity with in vitro assays. Further, within its CTD, our group identified that the specific chromatin binding ability is crucial for mitotic chromosome segregation. In this study, I investigated how the chromatin binding ability of TopoII contributes to the structural organization of mitotic chromosomes.

CRISPR/Cas9 genome-edited cell lines were created to allow for the investigation of TopoII mutants that have lost or altered chromosomal interaction domains. These cell lines eliminate endogenous TopoII and replace with the desired mutant form by the insertion of two drags. This system is critical because TopoII is an essential enzyme for mitosis thus conditional knockdown and inducible expression is required to test molecular function of TopoII in mitotic cells.

We have observed distinct protein pattern in isolated chromosomal scaffold fractions when TopoII is replaced to loss of chromatin binding mutants. Mass Spectroscopy analysis of the fractions will reveal proteins that are affected by TopoII binding to the chromosome. My study provided key discovery for the long-standing question of what role TopoII has as a component of the chromosomal scaffold.

40. Equipment and Services of the Kansas University Nanofabrication Facility

Ryan Grigsby, 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), atomic force microscopy, 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.

41. Bioactive Small Molecules Produced by The Gut Commensal Enterocloster citroniae Modulate Vibrio cholerae Behavior

Saeideh Nasiri, Heidi Pauer, Nathalia Santos Magalhaes, Luis Caetano M. Antunes

Microorganisms living in the human gut have long been known to protect their hosts against pathogens. Here, we focus on one gut microbiota species, Enterocloster citroniae, and demonstrate that extracts from pure cultures of this commensal dampen Vibrio cholerae motility. We also established infection protocols using V. cholerae and HT-29 cell lines to investigate the impact of E. citroniae extracts on V. cholerae interactions with host cells. Our data showed that E. citroniae inhibits V. cholerae adhesion to HT-29 cells. Also, E. citroniae extracts were tested for cytotoxicity and viability assays and showed that the compounds produced by E. citroniae are not cytotoxic. To

show the effects of the toxins produced by V. cholerae that is overnight grown in broth culture with E. citroniae extracts, viability assays were performed. 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. Preliminary data showed that the bioactive compound is <3 KDa. Mass spectrometry and nuclear magnetic resonance (NMR) assays will be performed to determine the chemical composition of active compound(s). To that end, we used 1H and 13C NMR to profile the chemical composition of extracts from active strains, and common features could be detected. Once compounds are purified and identified, we will investigate their effects on V. cholerae behavior. This work will shed light on V. cholerae interactions with its host and the associated microbiota through the lens of chemical biology.

42. Quorum Sensing Regulation by the ptsO and ptsN genes of the Nitrogen phosphotransferase system

Samalee Banerjee, Nicole Smalley, Josephine R. Chandler, Ajai Dandekar, Matthew Cabeen

The opportunistic pathogen, Pseudomonas aeruginosa, is a major cause of hospital-acquired infections and a significant burden in health care. P. aeruginosa uses the population-density dependent LasI-LasR quorum sensing system to regulate many key virulence factors. This system senses and responds to acyl-homoserine lactone signaling molecules, which are produced by LasI and detected by LasR, a transcriptional regulator that can activate expression of dozens of genes. P. aeruginosa virulence is also regulated by a nitrogen-related phosphotransferase system (PTSNtr), consisting of the PtsP, PtsO and PtsN proteins. PTSNtr is widely distributed in many pathogenic Proteobacteria and thought to be important for sensing and responding to carbon and nitrogen availability. We and others have demonstrated mutation of the first PTSNtr gene ptsP increases production of the toxin pyocyanin, which is regulated by LasI-LasR, as well as expression of lasI. The purpose of this study was to investigate the mechanism of PtsP-dependent activation of pyocyanin and the LasI-LasR system. The three PTSNtr genes were deleted singly and in combination and their effects on expression of quorum sensing-regulated genes was assessed. Our results showed that ptsP disruption increased expression of only a subset of quorum sensing-controlled genes: lasI, phzM (pyocyanin biosynthesis), and hcnA (hydrogen cyanide biosynthesis). Regulation of these genes was dependent on LasR and the LasI-generated signal provided endogenously or exogenously. Our results also showed that the other PTS-Ntr enzymes PtsN and PtsO also had regulatory effects on these genes. We constructed and tested mutations affecting predicted phosphorylation sites of the PTSNtr proteins, and our results were consistent with the idea that unphosphorylated PtsN caused gene activation while phosphorylated PtsO caused gene repression. We also provided evidence that PtsO and PtsN have distinct regulons, supporting that these two enzymes function independently. Our results provide new information about PTS-Ntr in P. aeruginosa and the role of this system in regulating key virulence factors in this important pathogen.

43. Integrating LigandMPNN into Rosetta

Samuel Lim, Joanna Slusky

With the growing usage of protein language models (PLMs), it becomes increasingly vital for biological tools and applications to adapt machine learning (ML) predictions for new and existing workflows. We examine current Rosetta-integrated PLMs and expand implementation coverage for ML models. We also build upon recent changes to the Rosetta computational suite and implement combined interfaces for ProteinMPNN (Dauparas, et al., 2022) and LigandMPNN (Dauparas, et al., 2023). Finally, we present an automated system for integrating MPNNs and other ML models into new workflows and applications.

44. Investigating Host-Viral Interactions in *Drosophila*: Insights from *Drosophila* innubila nudivirus (DiNV)

Taiye S. Adewumi, Robert Unckless

DNA viruses in *Drosophila* species offer valuable tools for studying host-viral interactions and hold significant potential as pivotal models for elucidating immune pathways and host-viral coevolution. However, naturally occurring DNA viruses infecting *Drosophila* species remain scarcely documented. The infectivity of naturally occurring DNA viruses like Drosophila innubila nudivirus (DiNV) across closely or distantly related *Drosophila* species, and the specific genes or gene responsible for interspecies resistance or susceptibility, remain largely unexplored. Furthermore, examining how different hosts respond to DiNV infection over time, and how the virus adapts to these hosts, can provide valuable insights into the coevolutionary relationships within a host-virus system. Here, I have outlined a comprehensive approach using systemic infection, quantitative PCR (qPCR), and genome sequencing to investigate viral infections in *Drosophila*. Nanoinjection facilitates precise viral DNA delivery for controlled infection studies. qPCR monitors viral load and host gene expression changes, while genome sequencing identifies genetic variation related to resistance or susceptibility and potential viral adaptation. This method provides a detailed understanding of host-virus interactions and the underlying genetic factors. The results present preliminary data from experiments involving viral (DiNV) infection across four *Drosophila* species; *Drosophila* innubila, *Drosophila* putrida, *Drosophila melanogaster*, and *Drosophila* affinis, as well as the adaptation of DiNV to S2 cells. This approach advances our understanding of evolutionary dynamics in host-virus interactions and highlights key genetic determinants of susceptibility.

45. RpoN-dependent phosphotransferase systems in Enterococcus faecalis

Tolulope I. Ade, Christian D. Decker, and Lynn E. Hancock

Phosphotransferase systems (PTS) play significant roles in the uptake and phosphorylation of sugars for metabolism. Enterococcus faecalis is predicted to encode 46 distinct PTS pathways, highlighting its versatility to grow on a variety of carbon sources, but many of the PTS substrates are unknown. In E. faecalis, six PTS are predicted to be regulated by the alternative sigma factor, RpoN, but only a small number of PTS substrates are known. To identify potential substrates for the RpoN-dependent PTS, we performed a Biolog carbon source phenotype array comparing commonly used strains of E. faecalis along with their isogenic rpoN mutants. We also took a bioinformatic approach to identify functionally characterized PTS from other organisms that show relatedness to the E. faecalis RpoN-dependent PTS. RpoN-dependent gene regulation in E. faecalis is also dependent on five bacterial enhancer binding proteins (bEBPs) of the LevR-family. Biolog results showed that the metabolism of glucose, mannose, cellobiose, gentiobiose, arbutin, salicin, glucosamine, and amygdalin require PTSs that are RpoN-dependent. Through bioinformatics, we were able to identify additional sugar substrates (glucosaminic acid, glucoselysine and fructoselysine) as potential PTS substrates dependent on RpoN. Through mutational analysis of the various bEBPs in E. faecalis, we were able to demonstrate a linkage between those sugars and a dedicated PTS responsible for their import. We also demonstrate through luciferase reporter assays that the PTS operons are induced by the sugar substrates in a manner that requires both RpoN and the corresponding bEBP.

46. Validation of a Proteoliposome γ-secretase Assay to Investigate Cholesterol's Effect on γ-secretase Function.

Tristan A. Sprague, Michael S. Wolfe

γ-secretase is a membrane protease that catalyzes the proteolysis of other membrane protein's helical transmembrane domains. γ-secretase is best known for its role in forming amyloid β peptide (Aβ) from amyloid precursor protein, a process that ultimately results in the amyloid plaques commonly found in the brains of patients suffering from Alzheimer's disease. γ-secretase also plays a central part in many other cellular processes, such as Notch signaling. Despite γ-secretase's important roles in cell function, relatively little is known about how the membrane's properties support γ-secretase function. We describe a newly revised proteoliposome-based assay system capable of investigating the relationship between the membrane and γ-secretase. We can incorporate γ-secretase and an APP-based substrate into the liposomes formed by our protocol, such that enzymatic activity is present only in the proteoliposomes after reconstitution. We also find that cholesterol increases γ-secretase activity differently in proteoliposome-based assays versus detergent-based assays and discuss some of the implications. This new method will enable a better description of how γ-secretase interacts with its lipid environment.

47. A genetic screen to identify mutations that modulate the effects of quorum sensing on antibiotic resistance in the bacterial pathogen Pseudomonas aeruginosa

Vanessa M. Schmidt, Brielle M. Mckee, Josephine R. Chandler

Pseudomonas aeruginosa is a multi-drug resistant pathogen that causes severe infections in immunocompromised hosts. Quorum sensing (QS), a cell-to-cell communication system involving a small diffusible signal and a signal-responsive transcription factor, regulates virulence and antibiotic resistance in P. aeruginosa and is a potential target for novel therapies. Paradoxically, QS-deficient mutants with mutations in the QS signal receptor lasR are frequently isolated from chronic P. aeruginosa infections in antibiotic-treated patients. Our previous work identified a mutation in the ribosome accessory factor EF-G1A that unexpectedly increased antibiotic resistance of lasR mutants, suggesting complex interactions between host adaptation and antibiotic resistance. My aim is to identify and characterize additional genetic mutations that interact with lasR to modulate antibiotic resistance in P. aeruginosa. We will employ two complementary approaches: transposon mutagenesis and long-term evolution experiments. A transposon mutant library will be generated in a P. aeruginosa signal synthase mutant (ΔlasI) and this pooled library will be screened for mutations affecting antibiotic susceptibility in a QS-dependent manner. Promising mutants will be further characterized using genomic, genetic, and biochemical approaches to elucidate the mechanisms underlying these interactions. This research will provide new insights into LasR function, P. aeruginosa physiology, and the evolution of antibiotic resistance in chronic infections. Our findings may inform the development of novel anti-infective strategies targeting quorum sensing in P. aeruginosa.

48. A novel model for neuroblast migration and differentiation: Lessons from QL neuroblast migration in Caenorhabditis elegans

Vedant Jain, Erik A Lundquist

Q neuroblasts are a pair of bilateral neuroblasts that are born in the posterior-lateral region of the animal, with QL on the left and QR on the right. Initially, QR migrates anteriorly over the V4 seam cell, whereas QL migrates posteriorly over the V5 seam cell. The second phase of migration is Wnt-dependent and begins after the first phase and the first Q cell division. In the second phase of migration, the QL cell encounters Wnt signaling that triggers the expression of a transcription factor called mab-5 which is both necessary and sufficient for posterior migration. Through RNA-Seq on early Q cells in mab-5 lof mutants, we have discovered a novel transcriptional cassette and

various epistatic interactions that are necessary for the second phase of migration. Our candidates include genes encoding for a variety of proteins like efn-4, vab-8,lin-17, and mab-20. EFN-4 and MAB-20 belong to the ephrin and semaphorin family of guidance cues. Whereas LIN-17 and VAB-8 are frizzled and kinesin proteins respectively. In addition to understanding the genetic pathway downstream of mab-5, in vivo live imaging of these cells at a single-cell resolution has led to a novel understanding of migration's genetic, morphological, and chronological underpinnings. Through this data, we propose a new and unconventional model for neuroblast migration at a single-cell resolution. In this model, we report for the first time that neuronal differentiation is triggered by changes in the biomechanical and biophysical properties of neuroblast and its surroundings rather than a complete transcriptional modification.

49. Small Molecule KRAS Inhibition in Colorectal Cancer

Alexa Magstadt, Andrew Evans, Dan Dixon

In the United States, colorectal cancer (CRC) is the third-most diagnosed and second-most lethal cancer among men and women combined. 30-40% of colorectal tumors contain a mutation in the small GTPase Kirsten Rat Sarcoma (KRAS). When KRAS is mutated and constitutively active, numerous signal transduction pathways implicated in cellular proliferation are activated. Due to KRAS's high mutation prevalence in CRC and role in tumor progression, it is an intriguing chemotherapeutic target. After years of being considered undruggable, recent advancements in binding affinity optimization have resulted in small-molecule therapeutics that allosterically suppress KRAS GDP/GTP cycling. MRTX1133 is one such drug that targets KRAS-G12D malignancies and is undergoing Phase 1/2 clinical trials. This work's focus is to characterize the specific effects of MRTX1133 treatment in KRAS-G12D CRC. In cell viability assays, MRTX1133 effectively induces cellular death in KRAS-G12D cells with an IC50 value in the nanomolar range. Furthermore, a 245-fold decrease has been observed between the IC50 values of KRAS wild-type and KRAS-G12D cells. The PI3K-AKT pathway's implication in this cell death has been demonstrated through western blotting and a phospho-kinase array. Further studies aim to increasingly understand MRTX1133's role in inducing cytotoxicity in KRAS-G12D CRC.

50. Using ^{19}F NMR to study the membrane interactions in the N-terminal domain of IpaB

Jacob Kroh

The Type III Secretion System (T3SS) is a complex nano-injector employed by infectious gram-negative bacteria, such as Shigella, Salmonella, Burkholderia, and Yersinia pestis. This system provides bacterial effector proteins a tunnel from the bacterial cytosol into the host cell through a pore it creates in the membrane. The T3SS is comprised of four highly conserved structures: the base, the needle, the tip complex, and the translocon. Though the individual component proteins may be structurally variable between species, the overall macrostructure and function are homologous. The atomic structure of the translocon is largely unknown. It is a heterooligomeric structure composed of a major and a minor translocase. In Shigella, these proteins are IpaB and IpaC, respectively. IpaB has a small ectodomain in its N-terminus that is ordered enough for a crystal structure to exist. Using this ectodomain, as well as a slightly larger truncation of the IpaB, our lab wanted to probe the potential for membrane interaction of this domain using 19F NMR. We found that there are indeed portions of this domain that interact with the membrane, as well as portions that do not. We then used these insights to create an interaction map of, and new working model for the membrane interaction of the N-terminal domain of IpaB. This information provides a novel glimpse into a potential mechanism for the insertion of IpaB into the membrane as well as better refinement of its topology around the host membrane-T3SS interface.