‘Wisc-e-sota’

4th Joint UMN-UW Virology Training Grant Symposium

October 6th, 2016 7-10pm

Opening reception & Keynote at Piggy’s Restaurant

October 7th, 2016 9am-4:30pm

UW-La Crosse, Cartwright Center

NIH T32-supported virology training programs at:

University of Wisconsin-Madison

University of Minnesota-Twin Cities

Featuring talks and poster sessions by students, postdocs and faculty

Sponsored by NIH grants T32 AI083196, T32 AI078985, and the Department of Microbiology at the University of Wisconsin-La Crosse

4th Annual *'Wisc-e-sota'* Virology Symposium

Thursday-Friday, October 6-7th, 2016
University of Wisconsin-La Crosse
Cartwright Center

Oral Presentations

Talk abstract numbers in left column

October 6th, 2016 (Thursday)

	6:30-7:00pm	Registration
	7:00-10:00pm	Opening Reception Piggy's 501 Front Street S, La Crosse, WI 54601 (608) 784-4877
	7:40-7:45pm	Welcome & Opening Remarks
	7:45-8:35pm	Opening Talk Bill Sugden American Cancer Society Professor, UW-Madison **"Keeping the tumor virus in the tumor cell: How EBV and KSHV do it!"**

October 7th, 2016 (Friday)

Convenor: Morgan Meissner

	8:30	Continental breakfast
1	9:00	Paul Ahlquist (UW) "**New structure / function insights into viral RNA replication compartments"**
2	9:30	Liang Guo, Jose Debes, Bernd Rattenbacher, Cavan Reilly, Daniel Beisang, Irina Vlasova-St. Louis, and Paul Bohjanen (UMN) **"Manipulation of host mRNA decay by hepatitis C virus"**
3	9:45	Jordan T. Becker and Nathan M. Sherer (UW) **"HIV-1 gag-pol mRNA subcellular distribution regulates sites of translation and virus particle production"**
	10:00-10:30	Morning coffee break and poster set up

Convenor: Tony Dawson

4	10:30	Luiza Mendonça, José O. Maldanado, Jessica L. Martin, Morgan E. Meissner, Sheng Cao, Wei Zhang, Louis M. Mansky (UMN) **"Cryo-electron microscopy analysis of HIV and HTLV ultrastructure"**

5	10:45	Megan E. Spurgeon, Mark Horswill, Johan den Boon, Omid Forouzan, Sonalee Barthakur, David Beebe, Avtar Roopra, Paul Ahlquist, and Paul F. Lambert (UW) **"Cell non-autonomous changes in stromal gene expression in a mouse model of HPV-associated cervical cancer"**
6	11:00	Julie K. Olson (UMN) **"Exosomes secreted by virus-infected microglia can activate bystander CNS resident cells"**
	11:30-2:30pm	Lunch, Poster Session & Symposium Group Picture
		Convenor: Amy Molan
7	2:30	Kristen Bernard (UW) **"Persistence of West Nile virus: a Goldilocks phenomenon"**
8	3:00	Beth K. Thielen, Hannah Friedlander, Sara Bistodeau, Kathy Como-Sabetti, David Boxrud, Anna Strain, Karen Martin, Ruth Lynfield (UMN) **"Influenza C virus associated with severe acute respiratory illnesses in Minnesota"**
9	3:15	Gloria Larson, Vy Tran, Shuĭqìng Yú, Yíngyún Caì, Jens H. Kuhn, and Andrew Mehle (UW) **"Genetic identification of the host factor PARP8 and ADP-ribosylation as suppressors of viral polymerase activity"**
		Convenor: Kathleen Makielski
10	3:30	Amanda Salzwedel, Chris LaRocca, Johee Han,J. Ryvlin, Julia Davydova, Masato Yamamoto (UMN) **"Oncolytic adenovirus expressing IFN-± synergistically potentiates chemotherapy, radiation, and chemoradiation in pancreatic cancer cells"**
11	3:45	James Romero-Masters, Reza Djavadian, Shidong Ma, Andrea Bilger, Eric Johannsen, and Shannon Kenney (UW) **"EBNA3C-deleted Epstein-Barr virus causes lymphomas in a humanized mouse model despite being severely deficient for B cell transformation in vitro"**
12	4:00	Yuying Liang (UMN) **"Immune evasion mechanisms of arenaviruses"**
	4:30	Closing remarks

Poster Presentations

Poster abstract numbers in the left column

1. "Analysis of Gag copy number and co-packaging in HTLV-1-like particles"
Wei Zhang, José O. Maldonado, Isaac Angert, Sheng Cao, Serkan Berk, Joachim D. Mueller and Louis M. Mansky

2. **"Role of ultraviolet radiation in papillomavirus-induced disease"**
Aayushi Uberoi, Satoshi Yoshida, Ian H. Frazer, Henry C. Pitot, Paul F. Lambert

3. "The phi29 connector crown domain is involved in DNA packaging"
Kristin Shingler, Selma Helal, Paul Jardine, and Shelley Grimes

4. "Nodavirus RNA genome length controls replication vesicle size, implying a new model for replication complex formation"
Desirée Benefield, Kenneth Ertel, Johan den Boon, Elham Ahmad, Marisa Otegui, and Paul Ahlquist

5. "Understanding HIV mutagenesis using high-throughput sequencing technology"
Morgan E. Meissner, Jonathan Rawson, Daryl Gohl, Kenny Beckman, Joshua Baller, Louis M. Mansky

6. "HIV frameshift site RNA stability correlates with frameshift efficiency and decreased viral infectivity"
Pablo Garcia-Miranda, Bayleigh E Benner, Jordan T Becker, Alexander Blume, Nathan M Sherer, Samuel E Butcher

7. "Investigation of bacteriophage phi29 ring motor through energy utilization assay"
Allen Eastlund, Shelley Grimes, Paul Jardine

8. "Regulation of APOBEC3B"
Amy Molan and Reuben Harris

9. "Novel post-integration species-specific barriers affecting persistent HIV-1 gene expression in non-human cells"
Evans III, E. L., Becker, J. T., Frickie, S., and Sherer, N.M.

10. "Development of an indirect enzyme-linked immunosorbent assay for the identification of antibodies to senecavirus A in swine"
Cheryl M.T. Dvorak, Zeynep Akkutay-Yoldar, Suzanne R. Stone, Steven J. Tousignant, Fabio Vannucci, and Michael P. Murtaugh

11. "HIV-1 and M-PMV RNA nuclear export elements program viral genomes for distinct cytoplasmic trafficking behaviors"
Ginger M Pocock, Jordan T Becker, Chad M. Swanson, Paul Ahlquist, Nathan M. Sherer

12. "Determinants of HTLV-1 CA-CA interactions involved in virus particle assembly"
Jessica L. Martin, Rachel Marusinec, Luiza Mendonca, Louis M. Mansky

13. **"Multimerization and membrane interactions of bromovirus 1a protein drive RNA replication complex assembly and function"**
Bryan S. Sibert, Janice Pennington, Amanda Navine, Paul Ahlquist

14. "Quantitative fluorescence imaging of single virus particles"
Isaac Angert, Ao Cheng, Yan Chen, Wei Zhang, Louis M. Mansky, and Joachim D. Mueller

15. **"HIV-1 Rev trafficking is regulated by multimerizaton-dependent NES masking"**
Ryan T. Behrens, Mounavya Aligeti, Ginger M. Pocock, Christina Higgins, and Nathan M. Sherer

16. **"The role of actin modulating GTPases in HIV-1 Env-induced cell-cell fusion"**
Jaye C. Gardiner, Nathan M. Sherer

17. **"Systemic challenge of newborn guinea pigs with cytomegalovirus results in structural and histological evidence of brain injury and reduced neurocognitive performance in a Morris water maze test"**
Claudia Fernández-Alarcón, Lucy Meyer, Jason C. Zabeli, Bradley C. Janus, Michael A. Benneyworth, Michael A. McVoy and Mark R. Schleiss

18. **"Codon usage bias and the natural history of cross-species viral transmission to African bats"**
Andrew J Bennett, Samuel D Sibley, Tony L Goldberg

19. **"Epstein-Barr virus infection of an oral keratinocyte model modulates the cell differentiation programs"**
Mark Eichelberg, Kyle McChesney, Dhananjay Nawandar, Shannon Kenney, Eric Johannsen

20. **"Using in vivo selection on a large scale HIV-1 Vif mutagenic library to delineate comprehensive binding interfaces of restrictive APOBEC3 proteins"**
Dan Salamango and Reuben Harris

21. **"Importance of CD8 T-cell responses targeting variable epitopes in SIV"**
Matthew Sutton, Amy Ellis, Alexis Balgeman, Gabrielle Barry, Andrea Weiler, Dane Gellerup, Hannah Schweigert, Thomas Friedrich, and Shelby O'Connor

22. **"Evaluating guinea pigs as an experimental model of congenital Zika syndrome"**
Craig J. Bierle, Claudia Fernández-Alarcón, Nelmary Hernandez-Alvarado, Jason C. Zabeli, Bradley Janus, Mark R. Schleiss

23. **"Identification of inhibitors of EBNA1 through high-throughput screening"**
Mitch Hayes, Ngan Lam, Bill Sugden

24. **"Genetic, structural, and in silico analyses of the APOBEC3F/Vif interface inform a wobble model"**
Christopher Richards, John S. Albin, Özlem Demir, Nadine M. Shaban, Elizabeth M. Luengas, Allison M. Land, Brett D. Anderson, Rommie E. Amaro, Reuben S. Harris

25. **"Influenza virus host range is regulated at the viral polymerase PB2:ANP32A interface where species-specific RNA synthesis defects control ribonucleoprotein assembly"**
Steven F. Baker and Andrew Mehle

26. **"Human papillomavirus promotes Epstein-Barr virus lytic reactivation in immortalized oral keratinocytes"**
Kathleen Makielski, Denis Lee, Laurel Lorenz, Dhananjay Nawandar, Ya-Fang Chiu, Shannon C. Kenney, Paul F. Lambert

27. **"The influenza virus polymerase anchors PKC delta to phosphorylate nucleoprotein and control progression through the viral life cycle"**
Anthony R. Dawson, Arindam Mondal, Gregory K. Potts, Joshua J. Coon, Andrew Mehle

28. **"An Epstein-Barr virus-encoded protein complex mediates late gene transcription from the newly replication DNA"**
Djavadian R., Chiu Y.F., Johannsen E.

Abstract – Opening Talk

“Keeping the tumor virus in the tumor cell: How EBV and KSHV do it!”

Adityarup Chakravorty, Ya-Fang Chiu, Kathryn Fox, Mitchell Hayes, Asuka Nanbo, Arthur Sugden, Dave Vereide, and Bill Sugden

Gamma-herpesviruses have evolved multiple strategies to exploit cellular machinery to support their genome-maintenance in cells. They also provide selective advantages to the infected cells to insure that cells that maintain their genomes outgrow those that lose them. KSHV (Kaposi's Sarcoma Herpesvirus) and EBV (Epstein-Barr Virus) both encode cis-acting origins of DNA synthesis and trans-acting origin-binding proteins to mediate their synthesis and partitioning. It has been established that EBV uses a discrete origin of DNA synthesis (DS), a separate maintenance element (FR), and the protein, EBNA1, which binds both elements for its plasmid synthesis. EBNA1 tethers EBV plasmids to chromosomal AT-rich DNA sequences directly to mediate quasi-faithful partitioning. Approximately 88% of its newly duplicated, sister plasmids are bound to opposite sister chromatids during S-phase and as such evenly divide between daughter cells. KSHV differs profoundly from EBV. Rather than binding to DNA sequences, KSHV uses 16 or more sets of replication origins located at the terminal region (TR) of its genome and a protein,LANA1, that binds the replication origins directly and tethers the KSHV genome to histones H2A and H2B in nucleosomes. We have examined KSHV to understand how the tethering of its genomes to nucleosomes via LANA1 mediates its segregation, an event essential to KSHV maintaining the tumors it causes. Live-cell imaging was combined with an independent, predictive computational model to uncover KSHV's unprecedented mechanism of segregation. KSHV not only tethers its genomes to nucleosome-bound chromosomal DNA but also to nucleosome-bound viral DNA to form clusters of genomes that partition as units. Super-resolution microscopy shows that these clusters are coherent aggregates not resolvable into their constituent plasmids. It shows also that KSHV plasmids are found in the LANA1 speckles detected by immunocytochemistry but not all of these speckles are associated with viral DNA. Clusters need to be tethered to chromosomes to be synthesized, too. We have uncovered the mechanism of cluster-formation by examining substitutions of LANA1 with moieties from EBNA1, which show that nucleosome-binding is essential for clustering. This cluster-forming mechanism confers a surprising distribution of plasmids in cells and an advantage to KSHV in establishing itself after infection. Clustering, as demonstrated computationally and observed in live cells, leads to a rapid establishment of high viral copy numbers in a population of cells.

Abstracts – Oral Presentations

1. "**New structure / function insights into viral RNA replication compartments"**
Paul Ahlquist

Positive-strand RNA virus genome replication occurs on dramatically remodeled intracellular membranes, often inside invaginated, vesicular replication compartments (RCs). We are dissecting RC structure, assembly and function to better understand and control virus replication. ~50 nm diameter, invaginated RC vesicles are formed on mitochondrial outer membranes by nodaviruses, which encode a single RNA replication protein A with RNA polymerase, RNA capping, mitochondrially-targeted transmembrane, and multiple essential multimerization domains. Prior to RNA synthesis, protein A recognizes defined cis signals to recruit viral genomic RNA to mitochondrial membranes. Intriguingly, subsequent RC vesicle formation requires viral RNA synthesis, mediated by an active protein A polymerase domain and additional cis-acting initiation signals on genomic RNA. Bromoviruses, in the alphavirus superfamily, similarly invaginate ER membranes to form ~70 nm RCs by the actions of viral protein 1a, which has RNA capping (1aN-Cap) and helicase-like (1aC-Hel) domains. 1a forms RCs by multimerizing, binding ER membranes, and recruiting viral RNA templates and 2aPol polymerase. 1a also recruits host factors, including membrane-shaping reticulons and ESCRT proteins essential to form RC vesicles. 1a primarily binds membranes through an amphipathic helix in 1aN-Cap, whose alternate 1a-1a and 1a-membrane interactions switch 1a between successive states controlling the order and balance of RC assembly steps. Ongoing mapping shows that 1a multimerizing interactions are complex, include mutually exclusive interactions implying alternate 1a conformations, and are essential for proper 1a localization, membrane remodeling, RNA template recruitment, and RNA synthesis.

2. "Manipulation of host mRNA decay by hepatitis C virus"

Liang Guo, Jose Debes, Bernd Rattenbacher, Cavan Reilly, Daniel Beisang, Irina Vlasova-St. Louis, and Paul Bohjanen

Manipulation of Host mRNA Decay by Hepatitis C Virus Liang Guo1, Jose Debes1, Bernd Rattenbacher1, Cavan Reilly2, Daniel Beisang1, Irina Vlasova-St. Louis1, and Paul Bohjanen1. 1Department of Medicine, Center for Infectious Diseases and Microbiology Translational Research, University of Minnesota, Minneapolis, MN, USA. 2Division of Biostatistics, School of Public Health, University of Minnesota, Minneapolis, MN, USA. Hepatitis C virus (HCV) establishes chronic infection by preventing host antiviral responses from inducing apoptosis of infected cells through mechanisms that are not completely understood. We identified a molecular mechanism through which the HCV non-structural protein 5A (NS5A) manipulates host mRNA decay to promote growth and prevent death of infected cells. Binding by NS5A to GU-rich sequences in viral RNA is required for viral replication. These HCV GU-rich sequences are similar to GU-rich elements (GREs), regulatory sequences found in the 3untranslated regions (3UTR) of host transcripts. GREs function as mediators of rapid mRNA decay and are found in numerous transcripts that encode regulators of cell growth and apoptosis. We hypothesized that NS5A binds to host GRE-containing transcripts and manipulates host gene expression by altering mRNA decay. We performed Actinomycin D mRNA decay assays followed by transcriptome-wide RNA sequencing on human hepatoma cells (Huh) and the same cells expressing a HCV subgenomic replicon (Huh-HCV) and identified host transcripts whose decay rates changed upon HCV infection. We also used cytoplasmic extracts from these cells to perform RNA immunoprecipitation with an anti-NS5A antibody followed by transcriptome-wide RNA sequencing of co-purified transcripts to identify NS5A target transcripts. We identified 942 out of 25017 host transcripts as NS5A targets. Among them, 701 are related to cancer, 294 are related to cell growth and proliferation, and 290 are related to cell death and survival. Furthermore, we discovered that GREs were enriched in the 3UTRs of NS5A targets, and GRE-containing NS5A targets were stabilized in Huh-HCV cells compared to control Huh cells. Since numerous GRE-containing transcripts encode proto-oncogenes and other proteins involved in regulating cell growth or apoptosis, HCV-induced stabilization of these transcripts could prevent death and promote growth of virus-infected cells, allowing the virus to bypass antiviral responses and establish a chronic infection. Over time, chronically infected cells that fail to die due to this viral manipulation of apoptosis pathways could acquire additional genetic damage, leading eventually to the development of hepatocellular carcinoma.

3. "HIV-1 gag-pol mRNA subcellular distribution regulates sites of translation and virus particle production"

Jordan T. Becker and Nathan M. Sherer

mRNA subcellular localization is a crucial but poorly understood aspect of viral replication cycles. For retroviruses such as HIV-1, full-length, unspliced genomic RNAs (gRNAs) serve both as mRNAs encoding the Gag/Gag-Pol capsid proteins as well as the genetic material packaged by Gag into virions that assemble at the plasma membrane (PM). The spatiotemporal regulation of Gag/gRNA interactions in the cytoplasm prior to assembly is only partially characterized. Here we used live cell imaging and functional assays to test the effects of modulating gRNA cytoplasmic abundance and gRNA subcellular distribution on Gag trafficking and the efficiency of virus particle assembly. Increasing the cytoplasmic abundance of full-length gRNAs mutated to no longer synthesize Gag had only minor effects on Gag assembly competency when provided in trans. By contrast, artificially tethering gRNAs competent for Gag synthesis to non-PM membranes or the actin cytoskeleton redistributed gRNA and Gag in the cytoplasm, induced aberrant sites of assembly, and markedly reduced virus particle production. The block to virus particle production was largely specific to Rev-dependent gRNAs, suggesting a model wherein spatiotemporal coordination of gRNA nuclear export, cytoplasmic diffusion, and localized gag/gag-pol mRNA translation at the PM are important to the integrity of the native viral assembly pathway.

4. "Cryo-transmission electron microscopic analysis of HIV and HTLV ultrastructure"
Luiza Mendonça, José O. Maldanado, Jessica L. Martin, Morgan E. Meissner, Sheng Cao, Wei Zhang, Louis M. Mansky

General knowledge of retroviral particle morphology among the retroviral genera is limited. Comparative virology is a powerful experimental approach for exploiting evolutionary biology to elucidate structure/function relationships. Using this approach, we have investigated differences in immature particle morphology among human retroviruses – particularly human immunodeficiency virus type1 (HIV-1) and type 2 (HIV-2) as well as human T-cell leukemia virus type 1 (HTLV-1). Intriguing differences have been discovered between the immature Gag lattice structure of these viruses. First, HIV-2 immature particles consistently show a complete and continuous Gag lattice structure, suggesting that the HIV-2 Gag lattice has an intrinsic curvature that contains less interspersed gaps than that reported for HIV-1. Second, the immature HTLV-1 Gag lattice includes regions in particles with a 'flat' lattice morphology, which is a novel morphological feature not observed in other retroviruses. Taken together, our observations to date highlight the importance of comparative virology in gaining new insights into how retrovirus particles assemble and how differences in Gag-Gag interactions likely result in morphological changes in immature particle structure.

5. "Cell non-autonomous changes in stromal gene expression in a mouse model of HPV-associated cervical cancer"
Megan E. Spurgeon, Mark Horswill, Johan den Boon, Omid Forouzan, Sonalee Barthakur, David Beebe, Avtar Roopra, Paul Ahlquist, and Paul F. Lambert

Human papillomaviruses (HPVs) infect epithelial cells and can consequently give rise to carcinomas at various anatomical sites including the cervix, anus, and oral cavity. Many recent studies have uncovered important roles for the stromal microenvironment in progression and maintenance of various epithelial cancers. Using our previously developed K14E6/E7 transgenic mice, in which the keratin 14 (K14) promoter specifically drives expression of the HPV oncogenes E6 and E7 to the stratified squamous epithelia, we sought to determine how epithelial expression of the HPV oncogenes affects gene expression in the surrounding microenvironment during cervical carcinogenesis. Laser capture microdissection was performed to isolate RNA from epithelial and proximal stroma regions of reproductive tracts harvested from non-transgenic and K14E6/E7 mice, which was then analyzed using whole-genome microarrays. Expression of HPV oncogenes in the murine cervical epithelium elicited significant changes in gene expression in the neighboring microenvironment. Gene ontology revealed that genes altered in the microenvironment by epithelial E6/E7 expression are involved in processes including DNA replication, cell cycle, proliferation, focal adhesion, and multiple catalytic and signaling pathways. In order to gain insight into genes that could function as paracrine signaling factors, we concentrated on genes upregulated in the stroma adjacent to HPV-positive epithelia that are classified as being in the extracellular space/region. Here, we observed a strong immune signature of genes that are involved in biological processes such as the inflammatory response, cytokine/chemokine activity, and neutrophil chemotaxis. One particular group of cytokines, the CXC chemokines (CXCLs) that are CXCR2 ligands, was significantly upregulated in response to epithelial E6/E7 expression and their expression was further increased in the microenvironment adjacent to cervical tumors. We have also observed an increase in chemokine expression in primary human cervical cancer epithelial cells when co-cultured with primary human cervical fibroblasts. We are currently pursuing both in vitro and in vivo methods to test the functional consequences of an activated CXCR2-CXCL signaling axis in cervical carcinogenesis. This study provides insight into the crosstalk between HPV-positive epithelia and the stromal microenvironment, which could ultimately be applicable to all HPV-associated cancers.

6. "Exosomes secreted by virus-infected microglia can activate bystander CNS resident cells"
Julie K. Olson

Exosomes are membrane-bound nanovesicles (30-100 nm) released from various cell types that can be taken up by other cells thus mediating intercellular communication. Exosomes can contain a wide variety of materials, including miRNA, mRNA, and proteins, that are specific to the cells from which they were released. Recently, we have determined that microglia infected with Theilers murine encephalomyelitis virus (TMEV) secrete exosomes. Microglia are the resident immune cells of the central nervous system (CNS) that originate from a myeloid origin. TMEV infection of susceptible mice leads to the persistent infection of microglia in the CNS. The persistent infection contributes to the development of a chronic progressive demyelinating disease associated with an inflammatory immune response in the CNS, similar to multiple sclerosis in humans. We have previously shown that TMEV infection of microglia activates an innate immune response with expression of type I interferons, cytokines, chemokines, and effector molecules associated with the inflammatory response in the CNS. Thus, we wanted to determine whether exosomes secreted by virus-infected microglia have an effect on bystander resident CNS cells, including uninfected microglia, astrocytes, and neurons. Our results showed that exosomes secreted from virus- infected microglia activated the innate immune response in bystander uninfected microglia, astrocytes, and neurons by increasing the expression of type I interferons and inflammatory cytokines. The exosomes secreted from TMEV-infected microglia contain viral RNA that could be transferred to bystander uninfected CNS cells. The viral RNA was sensed by innate immune receptors in the recipient cells and activated an innate immune response in the uninfected bystander cells. Most interestingly, exosomes secreted from microglia in the brain of TMEV- infected mice could activate an inflammatory immune response in the brain of uninfected mice, including activation of microglia, astrocytes, and neurons. These results show that exosomes secreted by TMEV-infected microglia contain viral RNA and can activate the immune response in bystander uninfected CNS cells which may contribute to the persistent virus infection and the inflammatory response associated with demyelinating disease.

7. "Persistence of West Nile virus: a Goldilocks phenomenon"
Kristen Bernard

West Nile virus (WNV) can lead to a persistent infection in humans and in several animal models. In mouse studies from our laboratory, WNV RNA persists in skin and central nervous system (CNS) tissues for 3 to 6 months post-inoculation (p.i.). Infectious virus is routinely isolated at 1 month p.i. and up to 4 months p.i. in some animals. This persistence occurs in the face of WNV-specific antibody and CD8+ T cell response in the CNS. We showed that the CD8+ T cells were impaired in the CNS, but not the spleen, and their function was partially restored upon blockade of IL-10R and/or PD-L1. These data suggest that the CNS microenvironment inhibits the CD8+ T cell response, resulting in minimal immune-mediated damage and delayed viral clearance – a balance that is "just right".

8. "Influenza C virus associated with severe acute respiratory illnesses in Minnesota"

Beth K. Thielen, Hannah Friedlander, Sara Bistodeau, Kathy Como-Sabetti, David Boxrud, Anna Strain, Karen Martin, Ruth Lynfield

Influenza C is generally thought to cause less severe, upper respiratory infections of children and young adults. These conclusions are largely based on cross-sectional serological studies and investigations of localized outbreaks. The epidemiology is not well described due to limited surveillance data and the lack of a sensitive, widely available diagnostic test. To address this knowledge gap, we implemented screening for influenza C as part of a surveillance network for acute respiratory illnesses (ARI) in Minnesota. Surveillance sites established in 4 outpatient clinics and 3 hospitals submitted clinical data and respiratory specimens from inpatients admitted for severe acute respiratory illness (ARI) and outpatients with influenza-like illness or ARI. Respiratory specimens (predominantly nasopharyngeal swabs and nasal washes) obtained from May 2013 through August 2016 were tested using a multiplex real-time RT-PCR for 23 respiratory pathogens, including influenza C. We tested 9654 specimens from inpatients and 1977 specimens from outpatients and identified 59 case-patients with at least specimen positive for influenza C, including 48 from inpatients and 11 from outpatients. Most detections occurred during the winter months (December-March). There was substantial year-to-year variability in number of detections, with 86% of detections in the 2014-2015 season. The median age of case-patients was 21 months (IQR 8 months-18 years); 56% were < 2 years old, and 24% occurred in adults (range 18-84 years). Patients with influenza C detections presented with cough (62%), respiratory distress (50%), fever (45%), congestion (43%), vomiting (27%), and wheezing (23%). Among hospitalized patients, the median length of stay was 2 days (IQR 1.5-4 days), and there were 5 intensive care unit (ICU) admissions. Medical co-morbidities were reported in 60% of hospitalized case-patients and all those admitted to the ICU. Asthma/chronic obstructive pulmonary disease (COPD) (19%) and prematurity (17%) and were the most common co-morbidities. At least one other respiratory pathogen was detected in 40 (69%) of case-patients, with rhinovirus/enterovirus, RSV and adenovirus being most common. Two of the 5 ICU patients had no other pathogen detected. The HEF gene was sequenced in 27 specimens from December 2014-April 2015, and both Kanagawa and Sao Paolo lineages were detected in inpatients and outpatients. In conclusion, with systematic testing using molecular assays, we detected influenza C in patients with severe and moderate illness. Most patients were young children, but detections also occurred in adults. Two lineages of influenza C had concurrent circulation. In the future, we plan to continue surveillance and molecular characterization of circulating influenza C viruses to further define the epidemiology of influenza C. Comparative studies of influenza C isolates in model systems may yield insights into the molecular determinants of pathogenesis.

9. "Genetic identification of the host factor PARP8 and ADP-ribosylation as suppressors of viral polymerase activity"

Gloria Larson, Vy Tran, Shuǐqìng Yú, Yíngyún Caì, Jens H. Kuhn, and Andrew Mehle

Influenza A virus (IAV), like all viruses, must co-opt host cellular machinery to support successful infection. To identify host factors that enhance or inhibit infection, we screened the NCI60 panel of cell lines. The NCI60 cell lines have well-characterized gene expression and proteomic profiles, enabling high confidence correlation analyses between gene expression and IAV susceptibility. We challenged the NCI60 panel with IAV, measured the differential susceptibility of each cell line, and performed gene correlation analysis. We identified several pro- and anti-viral host factors for IAV infection, including poly-ADP-ribose polymerase 8 (PARP8) as a potent anti-viral factor. Poly-ADP-ribose polymerases (PARPs) comprise a family of 17 proteins, most of which post-translationally modify target proteins with ADP-ribose (ADPr) by adding single ADPr moieties (mono-ADPr, MAR) or polymeric chains (poly-ADPr, PAR). ADP-ribosylation impacts various host cellular processes such as protein-protein interactions, cell division, and transcriptional regulation, and it is part of global stress responses. Moreover, MARylating and PARylating pathways have been shown to impact innate immune responses and the replication of multiple viruses, and catalytically inactive PARP13/ZAPL has recently been identified as an anti-influenza factor. We showed that IAV infection causes upregulation of global ADP-ribosylation in cells. Looking more closely at the RNP complex, we used affinity capture to demonstrate that NP and the polymerase are ADP-ribosylated. Specifically, PA and PB2, but not PB1 are directly modified with ADPr. Our systemic screen of human PARPs revealed that PARPs differentially associate with RNP components and modulate their ADP-ribosylation state. PARP8 and several others inhibited IAV polymerase activity in reporter assays, reduced viral gene expression, and decreased overall viral replication. These results indicate that ADP-ribosylation and PARPs modify the viral RNP and regulate the success of infection. Furthermore, because it is fully reversible, ADP-ribosylation may dynamically regulate IAV polymerase activity throughout the course of infection.

10. "Oncolytic adenovirus expressing IFN-± synergistically potentiates chemotherapy, radiation, and chemoradiation in pancreatic cancer cells"

Amanda Salzwedel, Chris LaRocca, Johee Han,J. Ryvlin, Julia Davydova, Masato Yamamoto

Aside from curative resection, there is no effective treatment against pancreatic adenocarcinoma (PDAC). Late diagnosis and high recurrence results in five-year survival of 7%. Notably, Phase II trials based on adjuvant therapy combining systemic IFN- ± (IFN) with radiation, 5-FU, and Cisplatin reported increase in five-year survival of 21%. Smaller Phase II trials adding Gemcitabine to the treatment protocol also showed 30% increase in the two-year survival of patients. Despite promising results, IFN trial drawbacks included high IFN systemic toxicity, and low IFN levels intratumorally. Drawbacks resulted in increased patient dropout, and as IFN is chemo-radio sensitizer, low IFN in tumors could have hampered therapy full potential. Aiming to improve efficacy and tolerability of the promising IFN therapy, we developed an oncolytic adenovirus expressing human IFN (5/3E3ADP-IFN or OAd-IFN). Vector has Ad5/3 fiber modification and overexpresses Adenoviral Death Protein, respectively contributing to increased infectivity and oncolysis in PDAC. Because Cox-2 is up-regulated in PDAC, Cox-2 promoter was included upstream of Adenovirus (Ad) E1 region, restricting OAd-IFN replication to PDAC. To achieve replication dependent expression of IFN, IFN gene was added to Ad E3 region under the control of major late promoter. To test vector in an immunocompetent syngeneic hamster model of pancreatic cancer, counterpart OAd vector expressing hamster IFN was generated (RGDE3ADP-ham-IFN or OAd-ham-IFN). RGD fiber modification was included to enhance vector infectivity in hamster cells. In vitro assays demonstrated that OAd-IFN increased cytotoxicity of chemotherapy (5-FU, Cisplatin, and Gemcitabine), radiation (4 and 8Gy), and chemoradiation (5-FU + radiation, Gemcitabine + Radiation, and 5-FU+ Cisplatin + radiation) in PDAC cells. Comparison between OAd-IFN and control vector not expressing IFN (OAd-LUC) indicated IFN expressed by OAd-IFN potentiated chemotherapy toxicity in PDAC cells. Also, combination index analysis showed that combinations of OAd-IFN or OAd-hamIFN with chemotherapy, radiation, or chemoradiation are synergistic. Also, combinations of OAd-IFN and OAd-hamIFN mimicking IFN clinical trial protocols were highly synergistic and cytotoxic. In vivo studies in hamster syngeneic model of pancreatic cancer showed that combinations of OAd-hamIFN with chemotherapy, radiation, and chemoradiation augmented tumor shrinkage, delayed tumor recurrence, and increased survival compared to non OAd-hamIFN treated groups. Quantification of viral replication showed OAd-hamIFN effectively replicates and spreads in tumors. Our data confirms IFN expressing OAd improves killing effect of chemotherapy, radiation, and especially chemoradiation in PDAC cells and tumors. Impressive synergistic interaction between OAd-IFN and chemotherapy, radiation, and chemoradiation suggests vector holds great potential to improve IFN therapy efficacy while decreasing its toxicity. As IFN therapy is of the few therapies reporting impressive improvement of pancreatic cancer survival, further development of OAd-IFN combinations mimicking IFN therapy is highly encouraged.

11. "EBNA3C-deleted Epstein-Barr virus causes lymphomas in a humanized mouse model despite being severely deficient for B cell transformation in vitro"

James Romero-Masters, Reza Djavadian, Shidong Ma, Andrea Bilger, Eric Johannsen, and Shannon Kenney

EBNA3C is an EBV-encoded latent protein essential for B cell transformation in vitro. A critical function of EBNA3C during EBV-mediated transformation in vitro is down-regulation of the tumor suppressor gene products, p14ARF and p16. EBNA3C also directly interacts with a cellular protein, IRF4, which promotes plasma cell differentiation of normal B cells and is an essential survival factor for activated B cell lymphomas. Here we have used a cord-blood humanized mouse model to examine the phenotype of an EBNA3C-deleted (EBNA3C) EBV mutant in vivo. We find that EBNA3C induces lymphomas in approximately 40% of animals, while wildtype (WT) virus induces lymphomas in virtually all animals. EBNA3C-induced lymphomas have a delayed onset (60-70 days) compared to WT tumors (30-40 days), but once present are as large as the WT tumors and highly invasive. Both WT- and EBNA3C-infected lymphomas are CD20-positive activated diffuse large B cell lymphomas that express IRF4. However, EBNA3C tumors express a higher level of BLIMP1 (a marker for plasma cell differentiation) than WT tumors. Furthermore, in comparison to WT tumors, EBNA3C tumors have increased numbers of lytically infected cells, consistent with enhanced plasma cell differentiation. EBNA3C tumors also have greatly enhanced expression of both p16 and p14ARF, as well as nuclear (wildtype) p53. However, EBNA3C tumors express high levels of Cyclin E, c-Myc, and BCL2, which may allow cells to bypass p16-mediated cell cycle inhibition and p53-induced apoptosis. Unexpectedly, we also find that EBNA3C tumors have greatly increased T cell infiltration in comparison to WT tumors, and show that T cell depletion of cord-blood humanized mice increases the ability of the EBNA3C mutant to cause lymphomas. Together, these results indicate that EBNA3C expression is not essential for EBV-induced lymphomagenesis in cord-blood humanized mice, and suggest that previously unappreciated roles of EBNA3C include inhibiting the T cell response to EBV-infected B cells, and blocking plasma cell differentiation.

12. "Immune evasion mechanisms of arenaviruses"

Yuying Liang

Several pathogenic arenaviruses, including Lassa virus (LASV), cause hemorrhagic fever (HF) infections that can result in significant morbidity and mortality in humans with limited preventative and treatment options. A hallmark of severe arenavirus HF is the high levels of viremia coupled with generalized immune suppression of the hosts, as evidenced by the impaired early innate immune responses, a delayed and weak humoral response, and the lack of CTLs. Understanding how pathogenic arenaviruses inhibit host immunity is key to develop effective interventions. Recent studies from my laboratory and others have revealed several unique immune evasion mechanisms of arenaviruses mediated by viral proteins such as the nucleoprotein (NP), the matrix Z protein, and the glycoprotein GPC. We have shown for the first time that the NP protein contains a 3252 exoribonuclease activity that is conserved among arenaviruses and is essential for viral replication by suppressing the IFN induction. We have recently uncovered a virulence-associated immune suppressive mechanism, in which the Z proteins of all known pathogenic arenaviruses, but not of non-pathogenic ones, can bind and inhibit the immune functions of RIG-i and MDA5. In addition, arenavirus GPC protein has been shown to evade the neutralizing activity of antibodies via glycan shield. Knowledge obtained from these studies will lead to the design of novel therapeutics against the deadly HF diseases and has been exploited for the development of new viral vaccine vectors.

Abstracts – Poster Presentations

1. "Analysis of Gag copy number and co-packaging in HTLV-1-like particles"

Wei Zhang, José O. Maldonado, Isaac Angert, Sheng Cao, Serkan Berk, Joachim D. Mueller and Louis M. Mansky

The Gag polyprotein is the main retroviral structural protein and is essential for the assembly and release of virus particles. In this study, we have analyzed the morphology and Gag stoichiometry of human T-cell leukemia virus type 1 (HTLV-1)-like particles and authentic, mature HTLV-1 particles by using cryo-transmission electron microscopy, scanning electron microscopy (cryo-EM) and fluorescence fluctuation spectroscopy (FFS). HTLV-1-like particles mimicked the morphology of immature authentic HTLV-1 virions. We observed that these virus-like particles (VLPs) has the unique local feature of a flat Gag lattice that does not follow the curvature of the viral membrane, resulting in an enlarged distance between the Gag lattice and the viral membrane. Co-transfection of various ratios of Gag and Gag-YFP were done to test the effects of the Gag-YFP on VLP morphology. We found that ratios of 3:1 (Gag:Gag-YFP) or greater resulted in HTLV-1- like particles that have a diameter, mass and Gag copy number similar to those in VLPs produced with the untagged HTLV-1 Gag.

2. "Role of ultraviolet radiation in papillomavirus-induced disease"

Aayushi Uberoi, Satoshi Yoshida, Ian H. Frazer, Henry C. Pitot, Paul F. Lambert

Human papillomaviruses are causally associated with 5% of human cancers. The recent discovery of a papillomavirus (MmuPV1) that infects laboratory mice provides unique opportunities to study the life cycle and pathogenesis of papillomaviruses in the context of a genetically manipulatable host organism. To date, MmuPV1-induced disease has been found largely to be restricted to severely immunodeficient strains of mice. In this study, we report that ultraviolet radiation (UVR), specifically UVB spectra, causes wild-type strains of mice to become highly susceptible to MmuPV1-induced disease. MmuPV1-infected mice treated with UVB develop warts that progress to squamous cell carcinoma. Our studies further indicate that UVB induces systemic immunosuppression in mice that correlates with susceptibility to MmuPV1-associated disease. These findings provide new insight into how MmuPV1 can be used to study the life cycle of papillomaviruses and their role in carcinogenesis, the role of host immunity in controlling papillomavirus-associated pathogenesis, and a basis for understanding in part the role of UVR in promoting HPV infection in humans.

3. "The phi29 connector crown domain is involved in DNA packaging"

Kristin Shingler, Selma Helal, Paul Jardine, and Shelley Grimes

The bacteriophage phi29 packages its dsDNA genome to near-crystalline density inside a preformed capsid shell using a powerful ATP-driven molecular motor. The packaging machinery includes pentameric pRNA and ATPase rings that transiently assemble at a unique capsid vertex delineated by the insertion of a dodecameric ring, referred to as the connector. These three concentric rings form a pore through which the phi29 genome is threaded. The crystal structure of the phi29 connector has been solved. The structure adopts a conical shape, with the wide end, comprised of the C-terminal portion of the protein, located inside the prohead and the narrow end, including the N-terminus, extending outwards. Three regions were not resolved in the crystal structure, including the C-terminal crown domain, channel loops that extend into the connector lumen, and the extreme N-terminus. Importantly, the crown domain and channel loops are predicted to be in contact with the virus genome. The disordered crown domain has been modeled. Residues 286-297 are predicted to form an ±-helix, while residues 298-309 are unstructured. Density corresponding to this domain is visible in the cryo-EM reconstruction, and appears to be in direct contact with the DNA. As the end-stage of packaging approaches the motor responds to the increasing load, modulating its mechanochemical cycle. Subsequently, the motor must terminate DNA translocation and dissociate from the capsid shell. These events require that the state of head filling be monitored and that the highly pressurized DNA be retained prior to tail assembly. The distal ATPase does not make contacts with the head shell, implying that the connector and/or pRNA must communicate the head-full state to the pentameric motor. We hypothesize that the connector crown domain functions as a pressure sensor based on the following observations: 1) the structure of the connector is altered during the phage maturation process, 2) connector mutants in other phage exhibit packaging defects, and 3) this region of the protein is located adjacent to both the actively translocating and packaged DNA. Two crown domain deletion mutations were introduced into the phi29 connector protein, a deletion of the unstructured region (”298-309) and a deletion of the entire C-terminus (”286-309). Both connector mutants form proheads that are indistinguishable from those formed with wild type connectors. In a highly efficient in vitro packaging assay the ”298-309 mutant packaged dsDNA at levels similar to wild type particles. The C-terminal deletion mutant packaged reduced levels of DNA. Proheads that incorporated connector proteins lacking the entire crown domain hydrolyzed twice the amount of ATP per DNA packaged as compared to WT connector proteins. The experimental data indicates that the connector crown domain is critical to the process of DNA packaging in the bacteriophage phi29.

4. "Nodavirus RNA genome length controls replication vesicle size, implying a new model for replication complex formation"

Desirée Benefield, Kenneth Ertel, Johan den Boon, Elham Ahmad, Marisa Otegui, and Paul Ahlquist

Flock House nodavirus (FHV) is an advanced model for genome replication by (+)ssRNA viruses, which include the Zika virus and many other pathogens. FHV RNA replication occurs on outer mitochondrial membranes in association with ~50 nm membrane invaginations termed spherules. Our recent cryo-electron microscope (cryoEM) tomography studies have revealed striking new insights into FHV spherule architecture, including that the spherule vesicles are tightly packed with filaments, matching our prior biochemical results that the apparently dsRNA FHV genome replication templates are protected in a membrane-associated state. In natural infections, FHV replicates two genomic RNAs, 3.1 kb RNA1 and 1.4 kb RNA2, and an RNA1-derived subgenomic RNA, 0.4 kb RNA3. Consistent with this range of template sizes, our cryo-EM tomography shows that infected cells contain filament-packed spherule vesicles with clearly distinct volumes ranging over at least 10-fold. Moreover, when we express the sole FHV RNA replication factor, protein A, with smaller sets of defined templates, the complexity and size range of FHV RNA replication vesicles changes accordingly. Prior results from our lab showed that before RNA replication, FHV protein A selectively recruits FHV RNA templates to mitochondrial membranes, but that spherule replication vesicles only form if viral RNA synthesis proceeds. Together with our new results that viral RNA template length controls replication vesicle size, these findings strongly suggest that the spherule replication vesicles form during initial synthesis of complementary viral (-)ssRNA, possibly by sequestering the growing dsRNA product into the growing vesicle. Moreover, our new cryoEM tomography results reveal a large, multimeric assembly induced by protein A that acts as a precursor to spherule formation. Overall, the results provide a dynamic new vision of RNA replication compartment formation, with likely implications for many other (+)ssRNA viruses and their control.

5. "Understanding HIV mutagenesis using high-throughput sequencing technology"

Morgan E. Meissner, Jonathan Rawson, Daryl Gohl, Kenny Beckman, Joshua Baller, Louis M. Mansky

Human immunodeficiency virus type-1 (HIV-1) infects approximately 35 million people worldwide and has killed over 39 million since its emergence in the human population. Treatment is difficult due to the rapid emergence of antiviral drug resistance as a result of high rates of viral mutation, which creates large genetic diversity not only between individuals, but within a single host. This diversity has frustrated decades of efforts for development of a successful vaccine. High rates of viral mutation are primarily driven by the low fidelity of the viral reverse transcriptase (RT) during replication, which results in an HIV-1 mutation rate near a theoretical error threshold, a rate beyond which increased mutagenesis would result in catastrophic collapse of the viral population. In addition to a high internal rate of mutation, HIV-1 is mutagenized by host factors known as APOBEC3 proteins in an attempt to restrict viral infectivity. The viral Vif protein, however, counteracts APOBEC3 activity and reduces this restriction to a low enough level such that host-mediated mutagenesis is also thought to contribute to viral diversification. Using high-throughput Illumina sequencing, our lab has sought to define the nature of viral diversity in HIV-1 populations, as well as apply this knowledge towards therapeutic approaches, with a goal of quenching HIV-1 infectivity through elevated levels of viral mutagenesis. Previous and ongoing projects within the lab have explored the use of nucleoside analogs to increase the rate of mutation in HIV-1, and have found that drugs such as decitabine and 5-azacytidine can act as potent viral mutagens, which may induce viral populations into a rate of lethal mutagenesis. In addition, we also seek to explore innate differences in mutation rates between HIV-1 subtypes and related HIVs, e.g. HIV type-2 (HIV-2), as a result of host elements such as APOBEC3 restriction factors and cell type. Our labs long-term goals are focused on characterizing the mechanisms of HIV mutagenesis, both in nature and in the context of novel therapeutic approaches.

6. "HIV frameshift site RNA stability correlates with frameshift efficiency and decreased viral infectivity"

Pablo Garcia-Miranda, Bayleigh E Benner, Jordan T Becker, Alexander Blume, Nathan M Sherer, Samuel E Butcher

HIV replication is strongly dependent upon a -1 programmed ribosomal frameshift to produce a gag-pol polyprotein precursor encoding essential viral enzymes necessary for production of mature virions. Here we investigate the relationship between the thermodynamic stability of the HIV-1 RNA frameshift site stem-loop, frameshift efficiency, packaging, and infectivity using pseudotyped HIV-1 and HEK293T cells. Our data reveal a strong correlation between frameshift efficiency and local, but not overall, RNA thermodynamic stability. Mutations that increase the local stability of the frameshift site RNA stem-loop structure increase frameshift efficiency 2-3 fold in infected cells, altering gag/gag-pol ratios. Thus, HIV- 1 frameshift efficiency is strongly affected by the strength of the thermodynamic barrier encountered by the ribosome. We further demonstrate that the surrounding genomic RNA secondary structure influences frameshift efficiency, and that a mutation that commonly arises in response to protease inhibitor therapy creates a functional, but inefficient secondary slippery site. Additionally, HIV-1 mutants with enhanced frameshift efficiencies are significantly less infectious and express altered ratios of packaged RNAs and capsid protein levels. This data suggests that compounds that increase frameshift efficiency by as little as 2-fold may be effective at suppressing HIV-1 replication. Finally, we hypothesize that altered frameshifting efficiency, corresponding with abnormal gag/gag-pol ratios, affects viral infectivity through changes to enzymatic activity and genomic RNA incorporation.

7. "Investigation of bacteriophage phi29 ring motor through energy utilization assay"

Allen Eastlund, Shelley Grimes, Paul Jardine

Bacteriophage phi29 utilizes a highly efficient pentameric ring motor for DNA packaging and exhibits remarkable coordination between consecutive subunits, requiring both up- and downstream communication. A working model of inter-subunit contacts places the trans-acting arginine finger in the dominant role, responsible for communicating the hydrolysis states to a subunits neighbors. It also is known to coordinate the exchange of ATP/ADP molecules, resetting the mechano-chemical cycle. Single molecule experiments have illuminated these mechanical steps in great detail but there remain several questions best investigated through ensemble experimentation. Point mutations made at key residues in and around the arginine finger have been screened using single molecule experiments and structural images to describe the assemblys role. Further ensemble investigation allows us to study the role of this trans-acting element at an kinetic level; how does the arginine finger regulate ATP/ADP exchange following processive translocation?

8. "Regulation of APOBEC3B"

Amy Molan and Reuben Harris

The APOBEC3 family of DNA-cytosine deaminases are well characterized as innate defense mechanisms against viral infection and are perhaps best known for their restriction of HIV-1. The APOBEC3s restrict HIV-1 by mutating proviral ssDNA cytosines to uracils disrupting the viral message. APOBEC3s have also been implicated in restriction of other viruses as well as transpositional elements and represent a critical part of the human innate immune system. As these defenders are themselves mutagens, what is to prevent them from turning against the cells they evolved to protect? Recent work has shown that for APOBEC3B, the regulatory mechanisms can fail and hypermutation of the human genome results. There is a multitude of evidence that APOBEC3B is regulated through its expression level, nuclear localization, cell type-dependent restriction of HIV-1. This poster will review recent work on the regulation of APOBEC3B.

9. "Novel post-integration species-specific barriers affecting persistent HIV-1 gene expression in non-human cells"

Evans III, E. L., Becker, J. T., Frickie, S., and Sherer, N.M.

Cells derived from mice and other rodents exhibit profound defects to HIV-1 virion production reflecting species-specific incompatibilities between the host dependency factors Cyclin T1 (CCNT1) and CRM1 and the viral Tat and Rev proteins, respectively. Whether CCNT1 and CRM1 represent the final remaining species-specific blocks affecting HIV-1s post-integration stages is unknown. In this study we monitored HIV-1 early and late gene expression in humanizedmurine 3T3 fibroblasts stably expressing HIV-compatible versions of CCNT1 and CRM1 (3T3.2H cells). Gag/Gag-Pol expression and virus particle production were rescued in the humanized mouse cell line, albeit with a delay to transcription of approximately 24 hours relative to human cells (HeLa). Remarkably, 3T3.2H cells were also highly tolerant of HIV-1 infection, persisting in culture indefinitely unlike human cells that experienced cytopathic effects within 48 hours of infection. We mapped this tolerance to the viral Vif accessory protein and its inability to induce G2/M cell cycle arrest in rodent cells. Taken together, these data demonstrate at least two remaining species-linked virus-host interactions affecting HIV-1s post-integration stages.

10. "Development of an indirect enzyme-linked immunosorbent assay for the identification of antibodies to senecavirus A in swine"

Cheryl M.T. Dvorak, Zeynep Akkutay-Yoldar, Suzanne R. Stone, Steven J. Tousignant, Fabio Vannucci, and Michael P. Murtaugh

Background: Seneca Valley virus (SVV), a member of the family Picornaviridae, genus Senecavirus, species Senecavirus A (SVA), is a recently identified single-stranded RNA virus closely related to members of the Cardioviruses genus. SVV was originally identified as a cell culture contaminant and was not associated with disease until 2007 when it was first observed in pigs with Idiopathic Vesicular Disease (IVD). Vesicular disease is sporadically observed in swine, is not debilitating, but is significant due to its resemblance to foreign animal diseases, such as foot and mouth disease, whose presence would be economically devastating to the United States. IVD disrupts swine production until foreign animal diseases can be ruled out. Identification and characterization of SVA as a cause of IVD will help to quickly rule out infection by foreign animal diseases.
Results: We have developed and characterized an indirect ELISA assay to identify serum antibodies to SVA. Viral protein 1, 2 and 3 (VP1, VP2, VP3) were expressed, isolated, and purified from E. coli and used to coat plates for an indirect ELISA. Sera from pigs with and without IVD symptoms as well as a time course following animals from an infected farm, were analyzed using this indirect ELISA to determine the antibody responses to VP1, VP2, and VP3. Antibody responses to VP2 were higher than VP1 and VP3 and showed high affinity binding on an avidity ELISA. The quantitative ELISA results were compared with an IFA assay, also in development, showing similar results.
Conclusions: This assay can now be used to help differentially diagnose IVD due to SVA and helping to quickly rule out the presence of economically devastating foreign animal diseases.

11. "HIV-1 and M-PMV RNA nuclear export elements program viral genomes for distinct cytoplasmic trafficking behaviors"

Ginger M Pocock, Jordan T Becker, Chad M. Swanson, Paul Ahlquist, Nathan M. Sherer

Retroviruses encode cis-acting RNA nuclear export elements that override nuclear retention of intron-containing viral mRNAs, including the full-length, unspliced genomic RNAs (gRNAs) packaged into assembling virions. The HIV-1 Rev-response element (RRE) recruits the cellular nuclear export receptor CRM1 (also known as exportin-1/XPO1) using the viral protein Rev, while simple retroviruses encode constitutive transport elements (CTEs) that directly recruit components of the NXF1(Tap)/NXT1(p15) mRNA nuclear export machinery. How gRNA nuclear export is linked to trafficking machineries in the cytoplasm upstream of virus particle assembly is unknown. Here we used long-term (>24 h), multicolor live cell imaging to directly visualize HIV-1 gRNA nuclear export, translation, cytoplasmic trafficking, and virus particle production in single cells. We show that the HIV-1 RRE regulates unique, en masse, Rev- and CRM1-dependent burst-like transitions of mRNAs from the nucleus to flood the cytoplasm in a non-localized fashion. By contrast, the CTE derived from Mason-Pfizer monkey virus (M-PMV) links gRNAs to microtubules in the cytoplasm, driving them to cluster markedly to the centrosome that forms the pericentriolar core of the microtubule-organizing center (MTOC). Adding each export element to selected heterologous mRNAs was sufficient to confer each distinct export behavior, as was directing Rev/CRM1 or NXF1/NXT1 transport modules to mRNAs using a site-specific RNA tethering strategy. Moreover, multiple CTEs per transcript enhanced MTOC targeting, suggesting that a cooperative mechanism links NXF1/NXT1 to microtubules. Combined, our comparative studies reveal two unanticipated mRNA nucleocytoplasmic transport behaviors programmed by contrasting mRNA nuclear export factors. In other words, mRNA export elements not only govern gRNA nuclear export but also pre-program gRNAs for distinct trafficking behaviors in the cytoplasm. Further elucidation of the molecular details underpinning these unique trafficking behaviors may inform the development of antiviral strategies targeting viral gene expression and/or infectious virion assembly.

12. "Determinants of HTLV-1 CA-CA interactions involved in virus particle assembly"
Jessica L. Martin, Rachel Marusinec, Luiza Mendonca, Louis M. Mansky

The retroviral Gag protein is the main structural protein responsible for virus particle assembly and release. The Gag protein alone in most instances is sufficient for production of virus-like particles (VLPs). The Gag proteins of human immunodeficiency virus type 1 (HIV-1) and human T-cell leukemia virus type 1 (HTLV-1) both have structurally conserved capsid (CA) domains, sharing a ²-hairpin turn and a centralized coiled-coil-like structure of six ± helices in the CA amino-terminal domain (NTD) as well as four ± helices in the CA carboxy-terminal domain (CTD). For both retroviruses, CA drives Gag oligomerization, which is critical for the formation of an immature Gag lattice structure requisite for the formation of VLPs. It has previously been shown that the HIV-1 CA CTD is a primary determinant for CA-CA interactions. A previous study with HTLV-1 suggested roles for both the CA NTD and CA CTD in Gag-Gag interactions and particle formation. In this study, we sought to more clearly define the role(s) of the HTLV-1 CA CTD and CA NTD in Gag-Gag interactions and in particle release. To do this, we first created a panel of four chimeric Gag constructs in which the CA subdomains were interchanged between HIV-1 and HTLV-1, including HIV-1 Gag with the HTLV-1 CA CTD (HIV-HT-CTD) and HTLV-1 Gag with the HIV-1 CA NTD (HTLV-HI-NTD). The subcellular distribution and protein expression levels of the chimeric Gags were analyzed by confocal microscopy and immunoblotting, respectively. The morphologies of VLPs were also investigated by cryo-electron microscopy. The subcellular distribution of HTLV-HI-NTD and HIV-HT-CTD Gag proteins appeared diffuse and non-punctate, and VLP production was abrogated. Our observations indicated that the HIV-1 CA CTD could functionally replace the HTLV-1 CA CTD, but that the HIV-1 CA NTD could not functionally replace the HTLV-1 CA NTD. To further investigate the HTLV-1 CA NTD, we conducted alanine-scanning mutagenesis within the loop domains. Site-directed mutants were screened for diffuse vs punctate Gag localization in cells as well as particle release, and this analysis identified 3 groups of mutants. Group 1 had WT phenotypes; Group 2 mutants had a diffuse localization in cells but produced particles; Group 3 mutants had a diffuse Gag localization in the cytoplasm but did not produce particles. Six key mutants (CAs containing alanines at residues 16-17, 17-18, 46-47, 47-48, 131-132, and 132-133) were identified in Group 3 and were further analyzed using in vitro methods. Specifically, we purified the HTLV-1 CA and analyzed the ability of WT CA and mutants to form oligomers via crosslinking and immunoblot analyses. Taken together, we have found that while structural and functional similarities exist in the CA domain of HIV-1 and HTLV-1 Gag, the HTLV-1 CA NTD is distinct in that key residues required for Gag-Gag interactions are located in this domain.

13. "Multimerization and membrane interactions of bromovirus 1a protein drive RNA replication complex assembly and function"

Bryan S. Sibert, Janice Pennington, Amanda Navine, Paul Ahlquist

All (+)RNA virus RNA replication occurs in membrane-associated replication complexes that are essential for many steps in viral replication. Replication complex assembly is a proven, valuable therapeutic target for (+)RNA viruses. To investigate how viral proteins target to and rearrange cellular membranes, we studied an advanced model system, brome mosaic virus (BMV). The multifunctional BMV 1a protein (961 aa) is the only viral factor necessary to induce replication compartment membrane rearrangements in its natural host and the model organism S. cerevisiae. Previous studies showed that the 1a N-terminal RNA capping domain (1aN) multimerizes into large protein filaments when expressed in vivo and is sufficient for localization to, and association with, endoplasmic reticulum membranes. To better understand the contributions of 1aN multimerization to conserved aspects of bromovirus RNA replication, we examined 1a function and RNA replication of the closely related cowpea chlorotic mottle virus (CCMV). We found that like BMV, CCMV 1a is capable of supporting RNA replication in yeast. Essential CCMV 1a functions, including 1a localization and replication complex assembly depended upon interactions required for 1aN multimerization, further supporting the importance of 1aN-1aN interactions for RNA replication. Using a series of hybrid BMV/CCMV 1a proteins we identified two highly conserved motifs in distal regions of 1aN whose compatibility is required for 1a localization. Further, co-immunoprecipitation analysis of BMV 1aN fragments, revealed two separate regions within 1aN capable of homo- and hetero- typic interactions. These data, and others, indicate that the earliest steps of replication complex assembly, 1a localization, membrane association, and multimerization, are likely linked and require contributions from throughout the 1aN domain. Our data support evidence that viral protein multimerization is essential for (+)RNA virus replication. BMV 1a shares significant structural and functional similarities with proteins in the alphavirus-like superfamily and other (+)RNA viruses. Thus, the interactions identified here may provide insight into RNA replication complex assembly of a broad range of viruses.

14. "Quantitative fluorescence imaging of single virus particles"

Isaac Angert, Ao Cheng, Yan Chen, Wei Zhang, Louis M. Mansky, and Joachim D. Mueller

Fluorescence microscopy is a commonly used tool to investigate labeled viral systems, allowing observation of viral assembly, fusion, and transport in live cell samples. Fluorescence techniques are typically employed along with qualitative or semi-quantitative observables. In many fluorescence experiments, deeper quantitative measures are accessible but rarely utilized because their reliability remains largely unknown. Additionally, quantitative fluorescence measurements promise broader applications in parallel with complementary techniques such as electron microscopy (EM). For example, the fluorescent intensity of labeled viral particles is related to the single-particle stoichiometry of labeled protein, which is difficult to measure through EM. Stoichiometry can be related to other features (such as diameter), which are easily measured by EM but generally not observable with fluorescence microscopy. However, a rigorous and well characterized method for converting fluorescence intensity to viral stoichiometry does not exist in literature. Additionally, the potential for confounding effects related to the optical system and the local environment of the fluorophore is rarely discussed. We propose an improved method to calculate viral particle stoichiometry from fluorescence images acquired on a two-photon microscope and discuss several challenges in the initial implementation of the technique. We present preliminary findings for several labeled viral systems including: 1. HIV-1 and HTLV-1 GagYFP virus-like particles, which produce surprisingly heterogeneous fluorescent intensities and 2. A Sindbis virus where the E2 glycoprotein is labeled, which produces more uniform particle intensities.

15. "HIV-1 Rev trafficking is regulated by multimerizaton-dependent NES masking"
Ryan T. Behrens, Mounavya Aligeti, Ginger M. Pocock, Christina Higgins, and Nathan M. Sherer

A challenge for eukaryotic gene expression is to maintain strong yet transient interactions between mRNA and protein transport receptors at the nuclear pore complex (NPC). The human immunodeficiency virus type 1 (HIV-1) encodes the Rev protein, a prototype for multimeric adaptor complexes that transiently link mRNAs to the host-encoded CRM1 nuclear export receptor. Rev mediates the directed transport of incompletely spliced viral mRNAs to the cytoplasm, which is essential for late viral gene expression and infectious virion production. To accomplish this, nuclear Rev multimerizes on a cis-acting viral mRNA structure, the Rev response element (RRE), and recruits Ran-GTP-bound CRM1 through Revs encoded leucine-rich nuclear export signal (NES). The cooperative assembly of the RRE/Rev/CRM1 complex imparts the avidity necessary for efficient viral mRNA nuclear export. Perturbing the interactions between these constituent factors remains an appealing target for novel antiretroviral therapeutics. We recently demonstrated that the addition of a second NES to the carboxy-terminus (NES2) of a functional Rev-mCherry fusion protein (Rev-2xNES) rescues a profound block to HIV-1 virion production in murine cells but has little effect on Rev function in human cells (Aligeti, Behrens et al., J Virol 2014 88(24):14207-21). This result is consistent with a model wherein multiple Rev/RRE interactions with CRM1 are needed for viral gene transactivation, and indicate that this activity is species-limited. Here, we exploited variants of the Rev-2xNES mutant to further study the role of NES number, context, identity, and strength in forming functional RRE/Rev/CRM1 transport complexes in human cells. Changes to NES strength and identity in the native (NES1) position were well tolerated in infectivity assays despite marked effects on Rev subcellular distribution and viral mRNA export dynamics. However, replacing NES2 but not NES1 with a supraphysiological NES (sNES) that binds with an abnormally high affinity to CRM1 drastically reduces virion production and yield a striking arrest of Rev/CRM1 complexes at the nuclear pore. Finally, we find that Revs ability to function despite a sNES in the native NES1 position is likely dependent upon Revs capacity to self-associate into homooligomers. Based on these data, we propose a model wherein Rev-Rev interactions direct the conditional masking of Revs native NES to control the recruitment of multiple CRM1 molecules to the RRE/Rev complex.

16. "The role of actin modulating GTPases in HIV-1 Env-induced cell-cell fusion"
Jaye C. Gardiner, Nathan M. Sherer

The Envelope (Env) glycoprotein of human immunodeficiency virus type 1 (HIV-1) plays multiple roles during the viral life cycle; acting as a fusion protein during viral entry and an adhesion molecule during cell-to-cell transmission forming virological synapses (VSs). How HIV Envs contrasting activities are regulated to strike a balance between VS turnover and syncytialization remains unclear. HIV Env-induced syncytia were recently documented in both humanized mice and HIV+ individuals and were found to be participants in viral spread in vitro. Using a novel two-color live-cell imaging approach, we show that removal of the N-terminal matrix (MA) domain of the structural polyprotein Gag significantly decreases Envs fusogenic potential at the VS. This decreased fusion was recapitulated by mutating a single amino acid in MA (L12E) that abrogates Env incorporation into budding particles. Env-induced cell-cell fusion was also sensitive to overexpression of dominant-negative forms of the Rho family of small GTPases, implicating peripheral actin dynamics in this process. Finally, increasing extracellular viscosity to levels more akin to those observed in vivo markedly increased cell fusion, potentially relevant to the high frequency of syncytia formation observed in infected tissues. By unraveling the determinants necessary to regulate Env mediated cell-cell fusion, our work provides insights into the cellular biology usurped during HIV infection.

17. "Systemic challenge of newborn guinea pigs with cytomegalovirus results in structural and histological evidence of brain injury and reduced neurocognitive performance in a Morris water maze test"

Claudia Fernández-Alarcón, Lucy Meyer, Jason C. Zabeli, Bradley C. Janus, Michael A. Benneyworth, Michael A. McVoy and Mark R. Schleiss

Congenital cytomegalovirus (cCMV) is a leading infectious cause of neurologic deficits in children. Vaccines against cCMV are a major priority. The purpose of this study was to explore the impact of systemic neonatal guinea pig cytomegalovirus (GPCMV) infection on brain histology, inflammation and neurocognitive function in guinea pigs using a Morris water maze model. Eight pups were inoculated by intraperitoneal route with a virulent, recombinant TurboFP635 red fluorescent protein (RFP)-expressing GPCMV within 96 hours of birth (5 x 106 PFU). Six pups were mock-infected. On days 15-19 post-infection (pi), the animals were subjected to testing in the Morris water maze to evaluate learning and memory. Viral load in blood and tissue was determined by qPCR. In addition, brain samples were collected to examine for histological abnormalities. Viremia was detected at day 3 pi in 7/8 experimentally infected animals. End-organ dissemination occurred in brain (1/8) lung (2/8), liver (7/8), and spleen (8/8). The experimentally infected animals showed brain damage evidenced by neuronal necrosis and dilated lateral ventricles. Double-stained sections with T-lymphocytes and GFAP markers revealed that areas of increased inflammatory cells were associated with a prominent reactive astrogliosis and that some cell clusters were surrounded by intensely stained GFAP-positive astrocytes. Iba1+ stained cells with highly branched processes were observed on five of the RFP GPCMV-infected guinea pigs, suggesting that these cells were activated microglia. Significant differences were observed between infected and uninfected pups in total distance traveled and escape latency ($p<0.0001$). Additionally, the infected animals crossed the target platform zone fewer times (0.89) than control animals (2.28; $p<0.05$). In summary, neonatal infection in guinea pigs produced brain damage, leading to significant cognitive and learning defects, as evaluated by histology and the Morris water maze. This model should prove valuable in evaluation of therapies and vaccines targeting prevention of neurodevelopmental sequelae caused by neonatal CMV infection.

18. "Codon usage bias and the natural history of cross-species viral transmission to African bats"

Andrew J Bennett, Samuel D Sibley, Tony L Goldberg

By studying codon usage bias, or the phenomenon in which synonymous codons are present in a genome at uneven frequencies, it is possible to identify the signatures of directed mutational pressure and translational selection in a gene. Viral codon usage bias may exhibit signatures of selection for translational efficiency in their natural host, and conservation of these signatures across host taxa may allow for the use of this viral genomic meta-datato provide insight into the evolutionary origin of a virus following cross-species transmission. It furthermore may help to identify novel pathways for viral transmission. This study utilizes the codon usage bias of a novel dicistrovirus infecting a hammerhead fruit bat (Hypsignathus monstrosus) from the Republic of Congo to identify a novel transmission pathway from insects to frugivorous bats.

19. "Epstein-Barr virus infection of an oral keratinocyte model modulates the cell differentiation programs"

Mark Eichelberg, Kyle McChesney, Dhananjay Nawandar, Shannon Kenney, Eric Johannsen

Epstein-Barr virus (EBV) is a human herpesvirus associated with the development of specific forms of lymphoma, nasopharyngeal carcinoma, and gastric carcinomas. Studies of EBV growth transformation of primary B lymphocytes into lymphoblastoid cell lines have led to rapid progress in our understanding of the virus's role in lymphomagenesis. Although carcinomas account for almost 90% of EBV associated cancers, progress has been limited due to lack of a physiologic in vitro model of EBV epithelial infection. Recently, Shannon Kenney's lab discovered that latent EBV infection could be established in telomerase immortalized normal oral keratinocytes (NOKs). NOKs differentiate in organotypic raft cultures and recapitulate the differentiation induced viral replication observed in vivo. Preliminary observations have indicated that EBV infection of NOKs interferes with the differentiation process. By staining EBV infected NOKs raft cultures, we observed disordered basal cell morphology and decreased staining for markers of late differentiation such as loricrin and filaggrin. EBV-associated nasopharyngeal and gastric carcinomas are often characterized by an undifferentiated phenotype, hypermethylation of tumor suppressor genes, and an absence of p53 mutations. We observed that NOK cells that had developed a spontaneous p53 mutation (K120E) exhibited a dramatic reduction in Notch signaling. NOK-EBV cells that did not have p53 activity also showed higher expression of the EBV oncogene LMP-1, which upregulates multiple cell survival factors. LMP-1 exhibits variable expression in NPC, with higher expression levels associated with more aggressive and metastatic tumors. Our observations of variable LMP-1 expression could thus offer insight into the expression of this oncogene in NPC. To further investigate how latent EBV infection interferes with NOK differentiation, we performed RNA-seq analysis comparing NOK to NOK-EBV. This revealed substantially different gene expression profiles, suggesting the perturbation of many cellular signaling processes. Preliminary evidence suggests that EBV microRNAs expressed during latent infection account for many of the observed effects on RNA expression. We are constructing EBV recombinants defective for microRNA expression that will be used to infect NOK cells to determine the extent to which these are responsible for EBV induced changes in epithelial expression. Mutants targeting other EBV gene products expressed in NOK-EBV cells also being tested. By studying the mechanisms by which latent EBV infection affects cellular processes such as differentiation in epithelial cells, we expect to elucidate the role played by EBV in carcinomagenesis and identify critical pathways that may be amenable to pharmacologic inhibition.

20. "Using in vivo selection on a large scale HIV-1 Vif mutagenic library to delineate comprehensive binding interfaces of restrictive APOBEC3 proteins"

Dan Salamango and Reuben Harris

The APOBEC3 family of cytosine deaminases is comprised of seven members, four of which are capable of suppressing HIV-1 viral replication. These four enzymes, APOBEC3D (A3D), APOBEC3F (A3F), APOBEC3G (A3G), and APOBEC3H (A3H) package into nascent viral particles and mutate cytosine to uracil in the newly synthesized viral cDNA. To counteract the mutagenic potential of these restriction factors, the HIV-1 genome encodes a viral protein called Vif. Vif is capable of binding these APOBEC3 enzymes and targeting them for proteasomal degradation via recruitment of the host-cell E3 ubiquitin-ligase machinery. Interestingly, the binding interfaces between Vif and the restrictive APOBEC3s seem to be somewhat distinct. Several studies indicate that the Vif-binding interface of A3F is similar to that of A3D, but is partially distinct from the binding interfaces of A3G and A3H. To gain a better understanding of the nature of these binding interfaces, I am utilizing an in vivo selection system coupled with Illumina next-generation sequencing (NGS) to examine hundreds-of-thousands of Vif mutants for their ability to differentially bind the restrictive A3s.

21. "Importance of CD8 T-cell responses targeting variable epitopes in SIV"
Matthew Sutton, Amy Ellis, Alexis Balgeman, Gabrielle Barry, Andrea Weiler, Dane Gellerup, Hannah Schweigert, Thomas Friedrich, and Shelby O'Connor

Eliciting effective CD8 T cells to combat HIV infection is complicated due to its enormous viral sequence diversity. While these cells can control virus replication, they also select for T cell escape variants. One rational approach to address this sequence diversity is to elicit CD8 T cells that target the most conserved viral regions. Currently, we are testing the hypothesis that CD8 T cells specific for invariant epitopes that do not accumulate escape variants are unable to control viral replication. We are testing this hypothesis with Mauritian cynomolgus macaques (MCMs) infected with wild-type SIVmac239"nef and a variant (SIV"nef-8x) with mutations in 10 CD8 T cell epitopes restricted by MHC alleles present on the M3 MHC haplotype. MCMs who are homozygous for the M3 MHC haplotype were infected with these viruses. We monitored viral load and T cell populations throughout infection and used IFNg ELISPOT assays to evaluate CD8 T cell responses. We infected 6 M3/M3 MCMs with SIV"nef-8x. Of those, 4 have controlled viral replication to under 1000 copies/mL over the course of 20 weeks. Interestingly, control of SIV"nef-8x was markedly delayed when compared to MCMs infected with wild-type SIVmac239"nef. Full proteome IFNg ELISPOT assays identified 6 newly targeted antigenic regions with the majority of these regions not accumulating variants through 8 weeks of infections. We have shown that CD8 T cell responses can be redirected towards previously unknown antigenic regions during acute infection and that a marked delay in the kinetics of viral control is observed. Our data suggests that targeting these new antigenic regions early in infection may have contributed to viral control. In addition, we have not observed variants within the majority of these regions over the course of infection, resulting in a theoretically appealing target for designing a CD8 T cell based HIV therapy.

22. "Evaluating guinea pigs as an experimental model of congenital Zika syndrome"
Craig J. Bierle, Claudia Fernández-Alarcón, Nelmary Hernandez-Alvarado, Jason C. Zabeli, Bradley Janus, Mark R. Schleiss

Zika virus (ZIKV) is an emerging flavivirus that causes severe birth defects, including microcephaly. ZIKV infections during pregnancy can cause spontaneous abortion and the virus is teratogenic, disrupting fetal neurodevelopment. Animal models that accurately recapitulate congenital Zika syndrome are urgently needed for vaccine development and for the study of ZIKV pathogenesis. Guinea pigs and humans have morphologically similar hemomonochorial placentas and the relatively long (~65 day) guinea pig gestation can be divided into trimesters that are developmentally analogous to those of humans. As guinea pigs have successfully been used to model transplacental infections by cytomegalovirus, syphilis, and Listeria monocytogenes, we sought to test whether ZIKV could cause infections with attendant fetal pathology during pregnancy. We found that guinea pig cells supported ZIKV replication in vitro. Experimental infection of non-pregnant animals did not result in overt disease, but pregnant guinea pigs infected early during the second trimester had reduced weight gain compared to uninfected dams or guinea pigs infected with cytomegalovirus. Pups born to ZIKV infected animals were significantly smaller than historic controls, and a robust antibody response against ZIKV was detected in both the pups and dams. These results suggest that the guinea pigs may be a valuable model of congenital ZIKV syndrome.

23. "Identification of inhibitors of EBNA1 through high-throughput screening"
Mitch Hayes, Ngan Lam, Bill Sugden

Epstein-Barr virus has been causally associated with several human cancers. Previous work in our lab has established that a subset of EBV-associated tumors is dependent on EBV. In these tumor cells, the forced loss of the viral plasmid results in the death of the cell by apoptosis. This effect is mediated in vitro via the inhibition of a single viral protein, Epstein-Barr Nuclear Antigen 1 (EBNA1) through the expression of a dominant negative protein. However, there are no existing small molecule inhibitors of EBNA1. We therefore undertook a high-throughput screening campaign to identify potential inhibitors of EBNA1. From an initial collection of more than 400,000 small molecules across multiple libraries, we identified less than 1 percent that showed inhibition of a cell based EBNA1 dimerization assay. Subsequent analysis for cytotoxicity, specificity, and several other factors reduced the candidates to two chemical series. The compounds in each of these series have demonstrated selective inhibition of EBNA1 activity, including the forced loss of EBV plasmids from tumor cells. We are currently working on transforming these candidates into validated leads for further development.

24. "Genetic, structural, and in silico analyses of the APOBEC3F/Vif interface inform a wobble model"
Christopher Richards, John S. Albin, Özlem Demir, Nadine M. Shaban, Elizabeth M. Luengas, Allison M. Land, Brett D. Anderson, Rommie E. Amaro, Reuben S. Harris

APOBEC3 enzymes are host factors that potently restrict viral replication by hypermutating viral reverse transcripts. More specifically, four members of this family APOBEC3D, APOBEC3F, APOBEC3G, and APOBEC3H are known to block replication of Vif-deficient HIV-1 through this mechanism. However, HIV-1 Vif antagonizes APOBEC3-mediated restriction by hijacking cellular host proteins to form an E3 ubiquitin ligase complex that ultimately degrades APOBEC3 proteins, allowing the virus to replicate. Advances in APOBEC3 and Vif structural biology are ongoing, however a molecular understanding of the APOBEC3/Vif interface remains elusive. Inter-species comparisons, virus adaptation experiments, and in silico studies were combined to understand the APOBEC3F-Vif binding interface and deduce a model that best explains all available genetic and biochemical data. Virus adaptation experiments revealed that a single amino acid derivative of human APOBEC3F (E324K) as well as rhesus macaque APOBEC3F, which naturally has K324, both selected for the emergence of a negative charge at position 71 in HIV-1 Vif (i.e., G71D). This compensatory charge swap to rescue viral infectivity was validated in singe-round and spreading infection studies, and then used as an anchor point for docking followed by molecular dynamics simulations to optimize the derived model. Strikingly, full 1 microsecond simulations revealed a long-lasting electrostatic interaction between APOBEC3F E289 and HIV-1 Vif R15, which was also validated by swapping charges and performing infectivity studies. These gain-of-function results highlight the importance of these particular residues and explain prior loss-of-function studies. Furthermore, these findings coupled with extant literature showing that the Vif-APOBEC3 interaction is functionally conserved across species, and that Vif uses distinct binding interfaces to selectively counteract different APOBEC3s (e.g. APOBEC3D/F/G/H), have prompted us to propose a Wobble Model that explains how Vif has evolved to form these separate, yet functional, contacts with APOBEC3 enzymes.

25. "Influenza virus host range is regulated at the viral polymerase PB2:ANP32A interface where species-specific RNA synthesis defects control ribonucleoprotein assembly"
Steven F. Baker and Andrew Mehle

Influenza A viruses rapidly adapt to their host environment and mode of transmission during infection. As a consequence, viruses can become specialized to a particular environment and restricted in their host range. This is especially true for the viral polymerase, where avian viral polymerases cannot function efficiently in mammalian cells. Rapid adaptation of avian polymerases during infection in mammalian cells selects for a single amino acid change in the polymerase PB2 subunit at position 627; the avian-signature PB2 E627 mutates to the human-signature K627 to permit replication in non-permissive cells. The pressure to select for this charge difference suggests host-specific regulation at the PB2 627 interface. ANP32A and ANP32B are host proteins that have been implicated in influenza replication and shown to promote vRNA synthesis in vitro. More recently, ANP32A has been identified as a species-specific host factor controlling influenza polymerase function. Avian hosts encode ANP32A that contains a unique 29-33 amino acid insertion that is necessary and sufficient to support replication of polymerases with PB2 E627. We have demonstrated that the insert-containing avian ANP32A rescues avian polymerases in mammalian cells in a dose-dependent manner. These rescued polymerases now support high levels of virus replication. Moreover, expression of avian ANP32A in mammalian cells enabled ribonucleoprotein particle assembly, the primary molecular defect associated with restricted polymerases. We showed that ANP32A associates with the viral polymerase in cells, and this interaction is enhanced in the presence of viral genomic RNA. Recombinant ANP32A directly interacts with the PB2 627 domain in vitro, which together suggests that RNA-bound polymerase conformations expose the PB2 627 domain to better support ANP32A binding. To query how the avian ANP32A insert regulates polymerase restriction, we tested species-specific interactions between avian or human-style ANP32A with polymerases containing E627 or K627 PB2. Our data suggest a checkpoint for polymerase adaptation to new hosts, where transient PB2:ANP32A interactions coordinate efficient RNA synthesis and ribonucleoprotein assembly.

26. "Human papillomavirus promotes Epstein-Barr virus lytic reactivation in immortalized oral keratinocytes"
Kathleen Makielski, Denis Lee, Laurel Lorenz, Dhananjay Nawandar, Ya-Fang Chiu, Shannon C Kenney, Paul F. Lambert

Epstein-Barr virus (EBV) and human papillomaviruses (HPVs) are human tumor viruses that cause head and neck cancers. Both viruses infect and replicate in upper aerodigestive tract epithelia. Some studies have detected co-infection in both oropharyngeal and nasopharyngeal cancers raising the possibility that these viruses could potentially affect each other's life cycles and/or oncogenic potential. Our lab has established an in vitro model system using organotypic raft cultures to test the effects of EBV and HPV on each other in stratified squamous oral epithelial cells. We found that the presence of HPV promotes EBV lytic reactivation. Specifically, HPV promotes EBV genome amplification and expression of an EBV immediate-early protein, Z. Studying the effects of HPV on EBV lytic reactivation could be a first step to determining if co-infection contributes to viral infection and head and neck carcinogenesis.

27. "The influenza virus polymerase anchors PKC delta to phosphorylate nucleoprotein and control progression through the viral life cycle"

Anthony R. Dawson, Arindam Mondal, Gregory K. Potts, Joshua J. Coon, Andrew Mehle

The influenza ribonucleoprotein (RNP) serves as the minimal unit required for viral gene expression and replication. Consequently, proper RNP formation is indispensable for influenza virus replication. The RNP is composed of the heterotrimeric polymerase bound to both ends of the nucleoprotein (NP)-coated genomic RNA. During RNP generation, monomeric NP oligomerizes to encapsidate nascent RNA produced by the viral polymerase. Perturbing NP oligomerization activity disrupts RNP assembly, leading to loss of viral gene expression and replication. We have previously shown that host-dependent phosphorylation of NP regulates its oligomerization and assembly of the RNP. Dynamic phosphorylation of NP is therefore necessary for successful replication. Yet, how NP phosphorylation is temporally regulated to enable gene expression early during infection and RNP assembly during genome replication later in infection remains poorly understood in part because the kinase responsible for NP phosphorylation is unknown. We showed that multiple protein kinase C (PKC) family members phosphorylate NP. We used knock out cell lines generated with the CRISPR/Cas9 system to demonstrate that PKC ´ primarily impacts RNP activity, with minor effects on viral entry. PKC ´ did not interact efficiently with its target NP, but rather is anchored by the viral polymerase to ensure phosphorylation and maintenance of monomeric NP. To understand how NP oligomerization is ultimately licensed at late times during infection, we performed a temporal analysis of the kinase-substrate interaction and phosphorylation of NP. Together, these data shed light on how the dynamic phosphorylation of NP controls progression through the replication cycle and identify a critical virus-host interaction as an appealing target for therapeutic intervention.

28. "An Epstein-Barr virus-encoded protein complex mediates late gene transcription from the newly replication DNA"

Djavadian R., Chiu Y.F., Johannsen E.

Epstein-Barr virus lytic replication is accomplished by an intricate cascade of gene expression that integrates viral DNA replication and structural protein synthesis. Most genes encoding structural proteins exhibit □true□ late kinetics □ their expression is strictly dependent on lytic DNA replication. Recently, the EBV BcRF1 gene was reported to encode a TATA box binding protein homolog, which preferentially recognizes the TATT sequence found in true late gene promoters. BcRF1 is one of seven EBV genes with homologs found in other ²- and ³-, but not in ±-herpesviruses. Using EBV BACmids, we systematically disrupted each of these □²³□ genes. We found that six of them, including BcRF1, exhibited an identical phenotype: intact viral DNA replication with loss of late gene expression. The proteins encoded by these six genes have been found by other investigators to form a viral protein complex that is essential for activation of TATT-containing reporters in EBV-negative 293 cells. Unexpectedly, in EBV infected 293 cells, we found that TATT reporter activation was weak and non-specific unless an EBV origin of lytic replication (OriLyt) was present in cis. Using two different replication-defective EBV genomes, we demonstrated that OriLyt-mediated DNA replication is required in cis for TATT reporter activation and for late gene expression from the EBV genome. We further demonstrate by fluorescence in situ hybridization that the late BcLF1 mRNA localizes to EBV DNA replication factories. These findings support a model in which EBV true late genes are only transcribed from newly replicated viral genomes.

RESIDENCE HALLS

- **1 Angell Hall - C2
- **2 Coate Hall - D2
- **3 Drake Hall - C3
- *4 Eagle Hall - E3
- **5 Hutchison Hall - C2
- 6 Laux Hall - E6
- *7 Reuter Hall - E6
- **8 Sanford Hall - E6
- *9 Wentz Hall - C3
- **10 White Hall - D6

OTHER FACILITIES

- 11 Anatomy & Physiology Annex - C5
- **12 Archaeology Center and Laboratories - B5
- 13 Baseball/Softball Fields - G3
- *14 Campus Child Center - D4
- *15 Cartwright Center - A5
- *16 Centennial Hall - B4
- *17 Center for the Arts - B3
- 18 Challenge and Ropes Course - F3
- *19 Cleary Alumni & Friends Center (Admissions Office) - E5
- *20 Cowley Hall of Science - C5
- *21 Graff Main Hall - A5
- *22 Health Science Center - C1
- 23 Heating Plant - B5
- 24 Hoeschler Tower - B4
- *25 Maintenance Building/ Campus Stores - G5
- **26 Mitchell Hall - B6
- *27 Morris Hall - A4
- *28 Murphy Library - C4
- *29 North Campus Field and Equipment Building - G4
- *30 Police Services - D5
- *31 Recreational Eagle Center - D3
- *32 Roger Harring Stadium at Veterans Memorial Field Sports Complex - C6
- 33 Student Center (under construction) - D5
- 34 Student Recreation Fields - G1, E7
- 35 Tennis Courts - D8
- 36 Veterans Memorial Field Sports Complex (football, practice, soccer, track and field) - D7
- 37 Veterans Memorial Monument - C6
- 38 West Chiller Plant (under construction) - C1
- **39 Whitney Center - D2
- *40 Wimberly Hall - D4
- *41 Wing Technology Center - A4
- **42 Wittich Hall - B5

*Handicapped accessible
**Limited accessibility