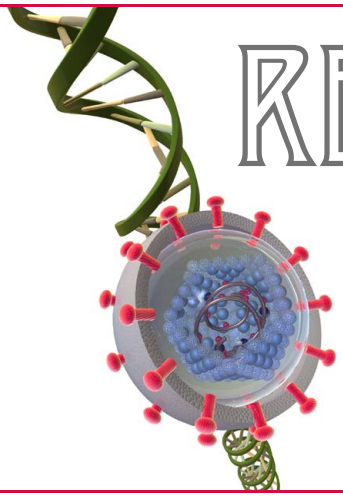

RETRO-ACTIVE NEWS

Newsletter of the Center for Retrovirus Research
at The Ohio State University College of Veterinary Medicine

2015 Highlights

The Center for Retrovirus Research 2015 Distinguished Research Career Award

Dr. Paul Bieniasz, PhD, Investigator, Howard Hughes Medical Institute, Aaron Diamond AIDS Research Center and Professor and Head, Laboratory of Retrovirology, The Rockefeller University was the 16th recipient of the annual award for his seminal contributions to our understanding of the molecular basis by which retroviruses parasitize host cell processes in order to replicate.

Dr. Bieniasz's research program addresses the conundrum that mammalian cells are well-equipped to inhibit virus replication, yet human retroviruses ultimately win the virus-host standoff. Using tools of molecular virology, genetics and genome-wide discovery, Dr. Bieniasz's research explores the basic mechanisms underlying retrovirus-host interactions, focusing on how HIV and related viruses replicate in cells. By identifying several host proteins necessary to promote or prohibit the replication of retroviruses, Dr. Bieniasz has taken strides toward understanding the molecular basis for HIV/AIDS.

In discovering the host defense molecule Tetherin, Dr. Bieniasz's research uncovered a previously unknown host activity: the formation of protein-based tethers that cause retention of fully formed HIV-1 particles on infected cell surfaces. Overcoming this restriction to spreading infection is Vpu, an HIV-1 encoded antagonist of Tetherin and thereby promotes the release and dissemination of HIV-1 particles from Tetherin-expressing cells.

The Bieniasz group determined ubiquitin ligases and components of the class E vacuolar protein-sorting pathway induce the fusion of virus and cellular membranes, promoting the release of nascent virus particles. The evaluation of HIV-1 RNA binding activity in cells and virions using cross-linking and RNAseq approaches revealed functional distinction in viral RNA binding activity: in the cytoplasm, Gag is bound to RNA sequences rich in guanine, but at the plasma membrane, Gag binding behavior changed to favor HIV's unusual adenine – rich sequence bias. Thus, Bieniasz's screening approach is unveiling the relationship of viral RNA sequence bias to assembly of infectious HIV-1.

Another antiretroviral protein, myxovirus resistance 2 (MX2), was identified by Dr. Bieniasz's comparative gene expression profiling of cells that differ in the activity of interferon-induced genes to inhibit virus replication. MX2 was identified to inhibit the ability of the virus to generate a provirus, the DNA form of the retrovirus, that is essential

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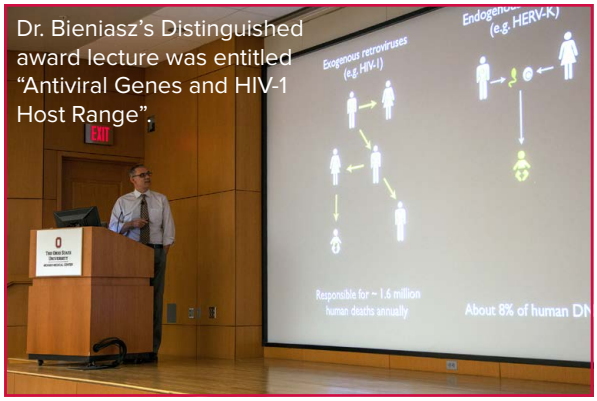
Dr. Paul Bieniasz (right) receives Career Award crystal from Center for Retrovirus Research Director Dr. Patrick L. Green (left).

The Center for Retrovirus Research 2015 Distinguished Research Career Award - continued from page 1



Dr. Bieniasz's Distinguished award lecture was entitled "Antiviral Genes and HIV-1 Host Range".

to complete the virus life cycle. MX2 inhibits HIV-1 infection by inhibiting capsid-dependent nuclear import of the subviral complex containing the reverse transcribed DNA.

Converting fundamental discoveries into practical benefits is an overarching goal of Dr. Bieniasz and his colleagues; in particular, they are working toward the goal of a better animal model of human AIDS. By engineering and adapting specific components of HIV-1 to avoid or inactivate the growing list of host restriction factors, including TRIM5 and APOBEC3, his group successfully derived simian cell tropic HIV-1 strain (stHIV-1). The generation of HIV-1 strains that can replicate in monkeys would be of enormous practical benefit and would likely revolutionize preclinical studies of HIV-1 drugs and vaccines.

Dr. Bieniasz's visit was sponsored by the Center for Retrovirus Research, Departments of Veterinary Biosciences and Molecular Virology, Immunology and Medical Genetics, Public Health Preparedness for Infectious Diseases Program, Center for Microbial Interface Biology, and the Comprehensive Cancer Center.

Dr. Karin Musier-Forsyth receives NIH/NIGMS RO1 to study RNA binding and packaging by retroviral Gag proteins.

Despite the vast excess of cellular RNAs, a single viral genomic RNA (gRNA) dimer is selectively packaged into new HIV-1 particles. The principal objective of Dr. Musier-Forsyth's study is to understand how selective gRNA packaging is achieved by Gag, a multi-domain protein that includes two nucleic acid-binding domains: matrix (MA) and nucleocapsid (NC).

Musier-Forsyth and other researchers in the field have shown that binding of the NC domain to the gRNA psi (ψ) packaging signal is necessary for efficient gRNA packaging, but the mechanism by which NC selectively interacts with psi is unclear. The role of the MA domain in cellular and viral RNA binding and regulation of specific gRNA packaging and membrane targeting has also been an active area of recent investigation in the field.

Musier-Forysth's work will address the many open questions that remain regarding the precise role of MA in selective gRNA packaging. These goals will be achieved by investigating the specificity of retroviral Gag binding to psi-containing and non-psi RNAs, and by probing the conformational dynamics, structure, and stoichiometry of HIV-1 Gag-RNA complexes.

Dr. Li Wu and Colleagues receive NIH award to explore the mechanisms that control HIV latency.

Dr. Li Wu, Professor in the Department of Veterinary Biosciences and Center member has received a grant from the National Institutes of Health.

HIV and TB infections are the world's most fatal infectious diseases, according to the World Health Organization. Across the globe, nearly 37 million people are currently living with HIV and in 2014, 1.2 million people died from AIDS-related illnesses. About 5,500 people contract HIV infection each day, but HIV basic research holds promise in eradicating this devastating disease.

The NIH grant will allow Dr. Wu and his team, which includes collaborators in China, to gather new information on the cellular and viral processes that underlie HIV's complex routes of transmission and replication. They will specifically focus on identifying currently unknown mechanisms that control HIV latency, which researchers have deemed "the most challenging question in HIV research." This study will facilitate the development of more effective strategies to combat HIV.

Dr. Mamuka Kvaratskhelia receives NIH/NIAID R01 for structure/ function studies of HIV-1 integrase.

HIV-1 integrase (IN) has an essential, multifunctional role in virus replication and serves as an important therapeutic target. HIV-1 IN catalyzes the covalent insertion of viral cDNA into the host chromosome. This process is mediated by cellular chromatin associated protein LEDGF/p75, which both markedly enhances integration efficiency and preferentially guides HIV-1 integration to actively transcribed genes. Structural interactions between HIV-1 IN and LEDGF/p75 have been well characterized, but until recently little was known about how LEDGF/p75 recognizes select chromatin sites.

Dr. Kvaratskhelia's research group has demonstrated that cooperative binding of the hydrophobic cavity and basic surface of LEDGF PWWP to the histone H3 tail containing trimethylated Lys36 (H3K36me3) and DNA wrapped in MNs is essential for high-affinity binding of LEDGF/p75 to chromatin. They now seek to characterize the interplay between full-length LEDGF/p75, H3K36me3 epigenetic marks, and HIV-1 integration sites in vitro and in infected cells. They will also test the hypothesis that HIV-1 IN plays an active role during the virus particle maturation.

The rationale for this notion was provided by observations that certain substitutions in the IN coding region or addition of allosteric IN inhibitors induce eccentric maturation of virus particles. However, little is known as to how IN could contribute to proper particle morphogenesis. The group's preliminary data indicates that HIV-1 IN interacts directly and with high affinity with highly structured viral RNA segments in vitro and in virus particles. These findings suggest previously undescribed roles of HIV-1 IN during virus particle maturation.

This study is expected to fill the critical gap in our knowledge of how multifunctional IN contributes to the late stage of HIV-1 replication.

Dr. Sanggu Kim, PhD joins the Ohio State faculty and the Center.

Dr. Kim became a tenure-track Assistant Professor in the Department of Veterinary Biosciences January, 2016. He joins us from UCLA, where he served as an assistant researcher under the mentorship of Dr. Irvin Chen.

Dr. Kim has formal education in both engineering and the life sciences, with a PhD in Biomedical Engineering (UCLA-2007). Dr. Kim's primary research goal is to establish a specialized translational program in which innovative multidisciplinary research tools are developed and employed to investigate the functional and genetic properties of hematopoietic stem cells (HSC), T cells, and HIV-1, with the ultimate goal of contributing to the development of gene therapies and other novel strategies to cure HIV/AIDS.

Dr. Kim also uses lentiviral vector tagging for genetic engineering of stem cells to study HSC and T cell behaviors *in vivo* at both the individual cell and entire system levels. Dr. Kim is the recipient of a NIH Pathway to Independence Award (K99/R00). Welcome Sanggu!

Congratulations!

Dr. Kathleen Boris-Lawrie departed Ohio State in August, 2015 to become chair of the Department of Veterinary and Biomedical Sciences at the University of Minnesota.

Congratulations Kathy and we wish you the best in this new endeavor.

Selected Grant Awards

Karin Musier-Forsyth
NIH RO1-GM065056 "RNA Binding and Packaging by Retroviral Gag Proteins" (2015-2019)

Li Wu
NIH/NIAID, R01AI120209 "HIV-1 Nef Interaction with Nef-associated Factor 1 Regulates Viral Latency Source" (2015-2018)

Li Wu
The Center for RNA Biology, Ohio State "Reversible m6A Methylation of HIV-1 RNA Regulates Viral Replication" (2015-2016)

Karin Musier-Forsyth
RO1-GM113656-01A1 "Translational Quality Control by Trans-editing domains (2015-2019)

Mamuka Kvaratskhelia
NIH/NIAID R01AI062520 "Structure and function of HIV integrase" (2015-2020)

Student, Post-doc and Research Scientist Awards

Feifei Wang (Wu Lab) – Oral Presentation Award, 2015 Hayes Graduate Research Forum

Rebecca Kohnken, DVM (Wu Lab) – NIH T32 Institutional Research Training Award

Alice Duchon (Musier-Forsyth Lab) - NIH F31 AI116396-01 "Mechanism of Human LysRS/ tRNALys Primer Recruitment and Packaging into HIV-1" (2015-2017)

Erik Olson (Musier-Forsyth Lab) - NIH F31 AI120868-01 "Mechanism of HIV-1 gRNA-Gag Interaction leading to selective genome packaging" (2016-2018)

Erik Olson, third place in the Biological Sciences for an oral presentation, 2015 Hayes Graduate Research Symposium

Selected Upcoming Meetings

Symposium on HIV/AIDS
March 3-5, 2016, Palm Springs, CA

Cold Spring Harbor Laboratory "Retroviruses"
May 23-28, 2016, Cold Spring Harbor, NY

American Society for Virology
June 18-22, 2016, Blacksburg, VA

10th International Retroviral Nucleocapsid Protein and Assembly Symposium
September 18-21, 2016, Montpellier, France

28th Workshop on Retroviral Pathogenesis
December 5-9, 2016, New Orleans, LA

2015 Graduates

Sarah Fritz, PhD (Boris-Lawrie Lab)
"Molecular basis of the DExH-box RNA helicase RNA helicase A (RHA/DHX9) in eukaryotic protein synthesis"
Accepted a Visiting Assistant Professor of Biology position at Antioch College, OH.

Alison Slaughter, PhD (Kvaratskhelia Lab)
"Mechanism of action of allosteric HIV-1 integrase inhibitors"
Accepted a position as a Patent Scientific Advisor, Philadelphia, PA.

Feifei Wang, PhD (Wu Lab)
"Comparison of Mouse and Human SAMHD1's Role in Retroviral Restriction and Cell Cycle Regulation"

Selected Publications

Tang C, Ji X, **Wu L**, Xiong Y. Impaired dNTPase activity of SAMHD1 by phosphomimetic mutation of Thr-592. *J Biol Chem.* 2015; 290(44): 26352-9.

Baydoun HH, Cherian MA, **Green PL**, Ratner L. Inducible nitric oxide synthase mediates DNA double strand breaks in HTLV-1 induced leukemia/lymphoma. *Retrovirology* 2015 Aug;12:71. doi: 10.1186/s12977-015-0196-y.

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Feng L, Larue R, Kessl JJ, **Kvaratskhelia M**. HIV-1 integrase multimerization as a therapeutic target. *Curr Top Microbiol Immunol*. 2015; 389:93-119.

Funderburg NT, Jiang Y, Debanne SM, Labbato D, Juchnowski S, Ferrari B, Clagett B, Robinson J, Lederman MM, McComsey GA. Rosuvastatin reduces vascular inflammation and T-cell and monocyte activation in HIV-infected subjects on antiretroviral therapy. *J Acquir Immune Defic Syndr*. 2015; Apr 1;68(4):396-404.

Kessl JJ, Sharma A, **Kvaratskhelia M**. Methods for the Analyses of Inhibitor-Induced Aberrant Multimerization of HIV-1 Integrase. *Methods Mol Biol*. 2015; 1354:149-64.

Kohnken R, Kodigepalli KM, **Wu L**. Regulation of deoxynucleotide metabolism in cancer: novel mechanisms and therapeutic implications. *Molecular Cancer*. 2015; 14(1):176.

Mates JM, de Silva S, Lustberg M, Van Deusen K, Baiocchi R, **Wu L**, **Kwiek JJ**. A Novel histone deacetylase inhibitor, AR-42, reactivates HIV-1 from chronically infected cells. *Retrovirology: Research and Treatment*. 2015; 7: 1–5.

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Panfil AR, Al-Saleem J, Howard CM, Mates JM, Kwiek JJ, Baiocchi RA, **Green PL**: Role of protein arginine methyltransferase 5 over-expression in HTLV-1-driven cellular transformation and leukemia. *Viruses* 2016; 8(1), 7; doi:10.3390/v8010007

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Senavirathne G, Liu J, Lopez MA, Hanne J, Martin-Lopez J, Lee J-B, **Yoder KE**, Fishel R. Widespread nuclease contamination in commonly used oxygen scavenging systems. *Nature Methods*. 2015; 12:901-902.

Singh PK, Plumb MR, Ferris AL, Iben JR, Wu X, Fadel HJ, Poeschla EM, Hughes SH, **Kvaratskhelia M**, Levin HL. LEDGF/p75 interacts with mRNA splicing factors and targets HIV-1 integration to highly spliced genes. *Genes Dev* 2015; 29(21):2287-97.

St. Gelais C, Roger J. **Wu L**. Non-POU domain-containing octamer-binding protein negatively regulates HIV-1 infection in CD4+ T-cells. *AIDS Res Hum Retroviruses*. 2015; 31(8):806-816.

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