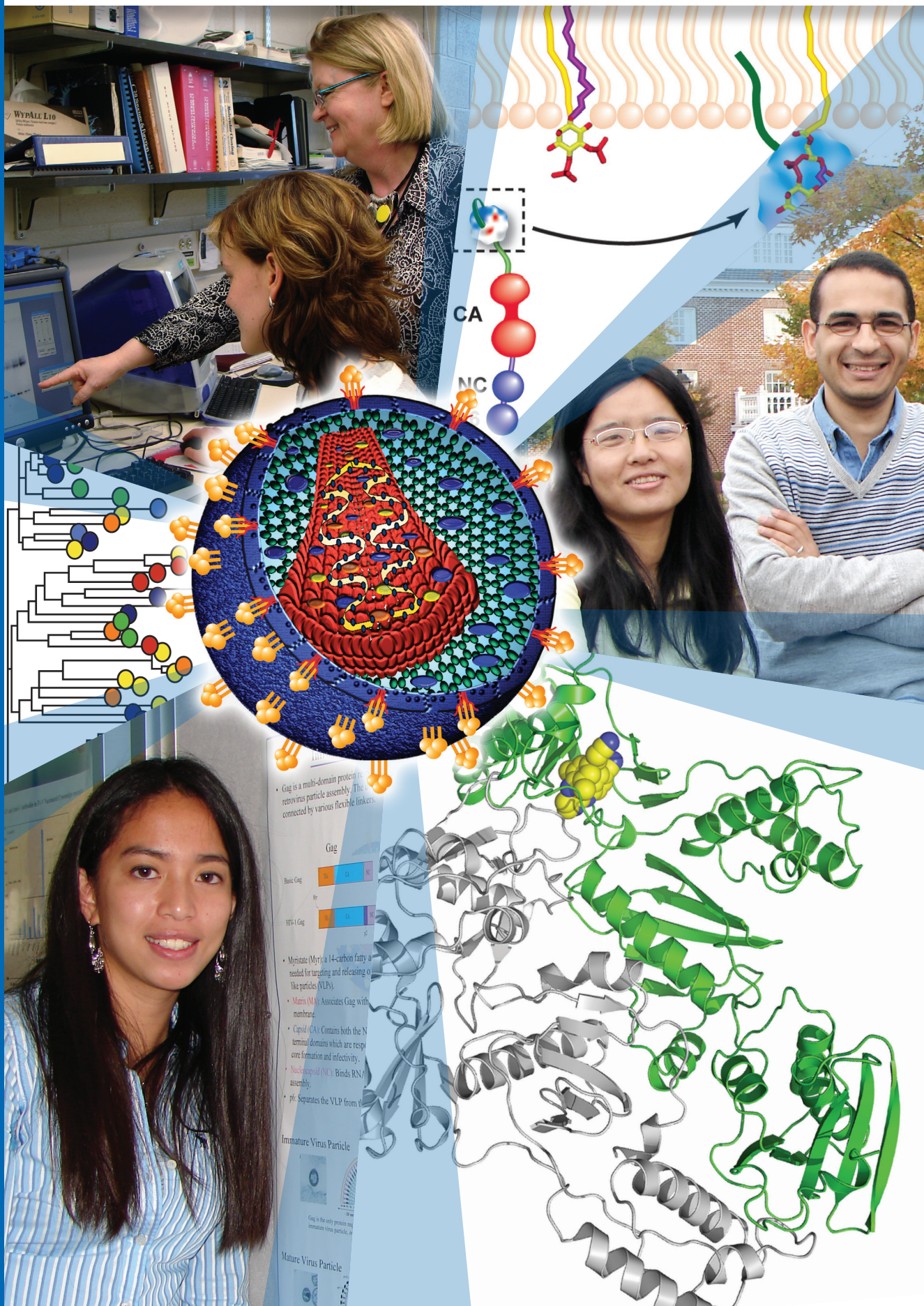
National Cancer Institute

HIV Drug Resistance Program



U.S. DEPARTMENT OF HEALTH AND HUMAN SERVICES

National Institutes of Health

Table of Contents

HIV Drug Resistance Program, National Cancer Institute

Directory ii

Message from the Director of the HIV Drug Resistance Program 1

Retroviral Replication Laboratory 3

- David Derse, Ph.D. 4
- Eric O. Freed, Ph.D. 6
- Wei-Shau Hu, Ph.D. 8
- Stephen H. Hughes, Ph.D. 10
- Vineet N. KewalRamani, Ph.D. 12
- Stuart F.J. Le Grice, Ph.D. 14
- Vinay K. Pathak, Ph.D. 16
- Alan Rein, Ph.D. 18
- Stephen Oroszlan, Ph.D. 20

Clinical Program — Host-Virus Interaction Branch 21

- Frank Maldarelli, M.D., Ph.D. 22
- Mary Kearney, Ph.D. 24
- Virology Core 25

Training and Employment Opportunities in the HIV Drug Resistance Program 26

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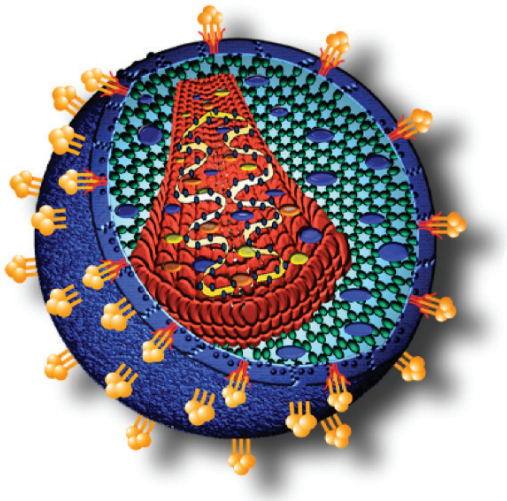
Message from the Director of the HIV Drug Resistance Program

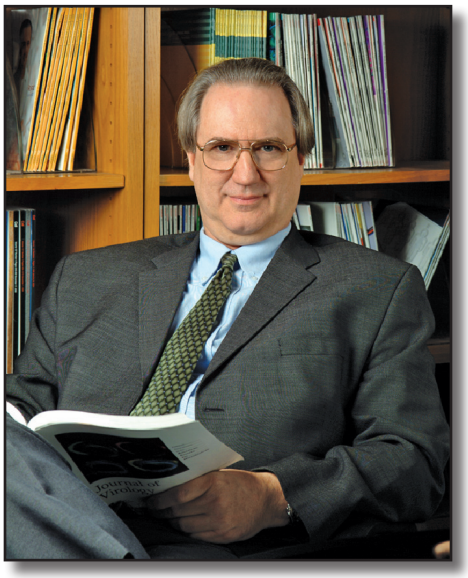
Approximately one million Americans and more than 30 million people worldwide are infected with HIV. Without effective therapy, virtually all will die of AIDS within 20 years. The real obstacle to successful therapy is the evolution of viruses that are resistant to the available antiviral drugs. At present, the only way to deal with this problem is with expensive, toxic, and strenuous multidrug regimens. Clearly, more research is needed to find new ways to deal with the resistance problem.

The HIV Drug Resistance Program (DRP) was initiated in 1997 with the mission of conducting and fostering multidisciplinary basic, translational, and clinical research focused on issues related to drug-resistant HIV at all levels. The Program has built on existing strengths of the National Cancer Institute's (NCI) basic and clinical research programs in retrovirology, drug discovery, and AIDS, exploiting structural biology, biochemistry, virology, virus-host interaction, evolution, and *in vivo* biology to accelerate drug discovery and better understand mechanisms of drug action and resistance. Not only are these areas of critical importance to the resistance problem, they also raise many basic research challenges whose solution will enhance our understanding of fundamental aspects of virology and cell biology. Although the Program focuses on HIV, resistance development is also a major concern with other infectious agents as well as cancer, and it is likely that our research will prove beneficial in these areas.



http://home.ncifcrf.gov/hivdrp

The DRP comprises the Office of the Director and research components that are divided into a basic and a clinical arm. The basic arm is the Retroviral Replication Laboratory, composed of research sections headed by eight Principal Investigators (PIs); the clinical arm is the Host-Virus Interaction Branch. These subdivisions operate as a cohesive group, with considerable interaction and collaboration among the researchers in the different components. Elements conducive to cohesion include biweekly senior staff meetings, often featuring chalk talks by one of the PIs; frequent work-in-progress meetings, in which Fellows present their ongoing work; and other joint events such as an invited speaker seminar series and biweekly meetings to discuss the clinical research with outside consultants Dr. John Coffin (Tufts University) and Dr. John Mellors (University of Pittsburgh).



Dr. Stephen H. Hughes

The DRP provides an important focus of HIV research within NCI and the National Institutes of Health as a whole — not only because of the quality and interactiveness of the individual researchers, but also because of the organized events the Program sponsors. These include the annual Symposium on Antiviral Drug Resistance (SADR), a four-day conference that the DRP co-sponsors with the University of Pittsburgh. The SADR series brings together researchers from all over the world and has become a very popular meeting in a very crowded schedule. Another major event is the annual Think Tank, a one-day meeting of NIH researchers interested in HIV, AIDS, and retrovirus research, which provides significant opportunities to learn about related work elsewhere on campus. Many valuable and productive intramural collaborations have been initiated

at this meeting. Periodically, the DRP has broadened participation in the Think Tank Meeting by inviting senior graduate students involved in retrovirus research outside the NIH to attend the Think Tank and to participate in an informal symposium with members of the DRP the following day.

All of the elements within the DRP — high-quality research, interactions and collaborations, and formal events — provide considerable added value to the NCI. DRP investigators are also an integral part of the NCI's Center of Excellence in HIV/AIDS & Cancer Virology (CEHCV), which was formed in 2006 to facilitate and rapidly communicate advances in the discovery, development, and delivery of antiviral and immunologic approaches for the prevention and treatment of HIV infection, AIDS-related malignancies, and cancer-associated viral diseases. Dr. Stuart Le Grice, a PI in the DRP, was appointed Head of the CEHCV. Through this Center of Excellence, we are working to enhance our interactions with both the intramural and extramural communities so that we can make the best use of the strengths and diversity within our Program and around us.

Stephen H. Hughes, Ph.D.
Director, HIV Drug Resistance Program
Center for Cancer Research
National Cancer Institute
National Institutes of Health

We are deeply saddened by the passing of our friend and colleague Dave Derse. We will remember him as a gifted scientist and special friend. Our deepest condolences go out to his family.

Retroviral Replication Laboratory

The Retroviral Replication Laboratory (RRL) focuses on obtaining a detailed understanding of important events in the retroviral life cycle with a strong emphasis on the human retroviruses HIV and HTLV. Members of the RRL study many aspects of the viral life cycle, from the early steps in infection of the host cell, through reverse transcription, to mechanisms of virus assembly and release. There is an increasing emphasis on the interactions of the host and the virus, and on the host factors that either aid or inhibit viral replication. These fundamental studies on the behavior of the virus and its interactions with the host cell are complemented by a strong commitment to understanding drug resistance. Studies also include discovery, development, and mechanistic analysis of novel replication inhibitors, as well as whole-organism studies, including the development of important animal models, and the development and use of retroviral vectors.

The RRL is composed of eight Sections:

- The Retrovirus Gene Expression Section, headed by **Dr. David Derse**, studies the replication of human T-cell leukemia virus (HTLV). HTLVs are complex pathogenic human retroviruses, but are only distantly related to HIV-1. Both types of viruses evade the immune system and are slow to cause disease; however, the underlying mechanisms are quite different.
- The Virus-Cell Interaction Section, directed by **Dr. Eric O. Freed**, is known for its work on the assembly and release of HIV-1 from infected cells and the host factors essential for efficient assembly and release. This Section has also been determining the mechanism of action of, and the development of resistance to, the Panacos maturation inhibitor PA-457.
- The Viral Recombination Section, directed by **Dr. Wei-Shau Hu**, focuses on mechanisms of recombination, RNA packaging, and virus assembly.
- The Vector Design and Replication Section, directed by **Dr. Stephen H. Hughes**, has two principal areas of research interest: 1) structural and biochemical studies of HIV-1 reverse transcriptase (RT); and 2) retroviral vectors.
- The Model Development Section, directed by **Dr. Vineet N. Kewal Ramani**, is primarily interested in understanding the role of host factors in HIV-1 infection and in developing an RT-SHIV that can be used to study anti-RT drugs and drug responses in a macaque model.
- The RT Biochemistry Section, under the direction of **Dr. Stuart F.J. Le Grice**, focuses on the biochemistry of HIV RT and related retroviral RTs, on the interaction of these RTs with their nucleic acid substrates, and on the development of RNase H inhibitors.
- The Viral Mutation Section, headed by **Dr. Vinay K. Pathak**, focuses on mechanisms by which APOBEC3 host restriction factors inhibit HIV-1 replication, interactions between HIV-1 Vif protein and APOBEC3 proteins, and how mutations in the connection subdomain and RNase H domain of HIV-1 RT confer resistance to antiviral RT inhibitors.
- The Retrovirus Assembly Section, led by **Dr. Alan Rein**, has been focusing on the roles of elements of the Gag protein, nucleic acid, and host factors in virus assembly.

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Research Interests:

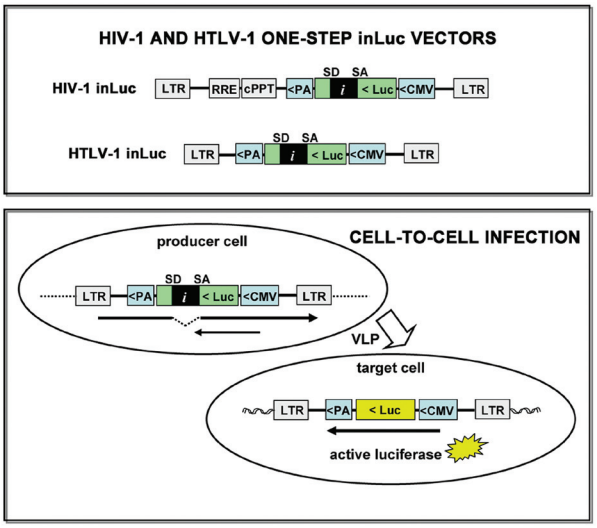
- Comparative retrovirology
- Mechanisms of HIV-1 and HTLV-1 infection and replication
- Control of cell and virus gene expression
- Viral pathogenesis and persistence

David Derse, Ph.D.

Head, Retrovirus Gene Expression Section, HIV Drug Resistance Program

Biography:

Dr. David Derse earned his Ph.D. in 1982 from the State University of New York at Buffalo, studying inhibitors of herpesvirus and cellular DNA polymerases in the laboratory of Dr. Yung-Chi Cheng. Dr. Derse did his postdoctoral research on bovine leukemia virus gene regulation under the supervision of Dr. James W. Casey at the Louisiana State University Medical Center. After joining the National Cancer Institute in 1986 as a Senior Staff Fellow, Dr. Derse continued research on the Tax and Rex proteins encoded by deltaretroviruses. In 1991, he became a tenured Senior Investigator and extended his research into comparative biochemical analyses of lentivirus Tat and Rev proteins. In 2004, Dr. Derse joined the HIV Drug Resistance Program as Head of the Retrovirus Gene Expression Section. He is also an Adjunct Professor in the Graduate Program in Genetics at George Washington University in Washington, D.C. Dr. Derse currently serves on the Editorial Board of *Retrovirology* and the Executive Committee of the Center of Excellence in HIV/AIDS and Cancer Virology, Center for Cancer Research. His current work focuses on the molecular mechanisms of retrovirus infection, replication, and host cell restriction.

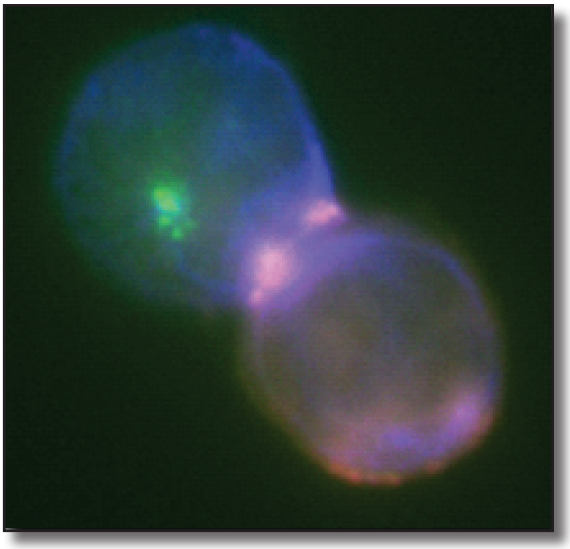


A specialized type of retroviral transfer vector has been constructed to facilitate studies of cell-to-cell infection for HIV-1 and HTLV-1. The upper part of the figure shows HIV-1 and HTLV-1 inLuc reporter vectors. In addition to the viral long terminal repeats and elements necessary for viral mRNA packaging and replication, the vectors contain an inverted reporter gene cassette. The cassette is composed of a CMV promoter, a luciferase gene containing a γ-globin intron oriented in the opposite direction, and a poly A signal. As shown in the lower part, when co-transfected with viral packaging plasmids, the spliced viral genomic mRNA is packaged into virus-like particles (VLPs) that infect target cells and generate a provirus capable of producing luciferase. The reporter protein is expressed exclusively in target cells after reverse transcription and integration, making it possible to quantify virus infection in a coculture setting.

Research Summary:

Although divergent retroviruses share a common mechanism for genome replication, they have evolved distinctly different strategies for infectious transmission, evading host immune responses, and resisting intrinsic cellular defense mechanisms. These variations are likely to reveal important insights into cellular pathways that viruses hijack to ensure their propagation and persistence, and reveal how certain host-virus interactions are related to pathogenesis. In this light, it is useful to compare HIV 1 with other retroviruses, in particular HTLV-1. Both of these human retroviruses target T cells for infection *in vivo* but with distinctly different disease outcomes. Whereas HIV-1 causes AIDS in almost all infected individuals, HTLV-1, which infects approximately 20 million people worldwide, is associated with adult T-cell leukemia and a degenerative inflammatory disease of the central nervous system in about 5% of infected patients. We are comparing the molecular and cellular biology of HIV-1 and HTLV-1 to understand fundamental mechanisms for cell-to-cell transmission of retroviruses, mechanism of action of APOBEC3 restriction factors, mechanisms of virus resistance to restriction, and mechanisms of virus protein trafficking essential for assembly of infectious virus particles.

To study mechanisms of cell-to-cell infection, we have developed special HIV-1 and HTLV-1 vectors and quantitative infection methods that will complement existing imaging technologies. These new vectors and cell culture model systems now make it possible to examine infection in a coculture setting. Combined with biochemical and microscopic image analysis, the new methods are helping to define how polarized virus assembly, release, transfer, and entry are coordinated with adhesion-induced changes in cell morphology.



HTLV-1 Gag (magenta) is mobilized to sites of contact between HTLV-1-transfected and nontransfected Jurkat T cells. Cells were stained with fluorescent-labeled antibodies that recognize HTLV-1 Gag (magenta), HTLV-1 Tax (green), and alpha-tubulin (blue).

We are using a comparative approach to better understand the mechanism of retrovirus restriction by APOBEC3 proteins. HIV-1 counteracts the antiviral activity of human A3G by producing an accessory protein, Vif, which hastens the degradation of A3G. In contrast, HTLV-1 encodes a peptide motif in the C-terminus of the HTLV-1 nucleocapsid domain that inhibits A3G packaging into nascent virions. Studies of HIV-1, HTLV-1, and other retroviruses are helping to elucidate how A3G is incorporated into virions, how it inhibits virus replication, and how viruses resist its antiviral effects.

We are also examining mechanisms of Gag targeting to specific membrane microdomains, envelope trafficking, regulation of Env activity, and the integration of Gag and Env trafficking pathways involved in retrovirus assembly and release.

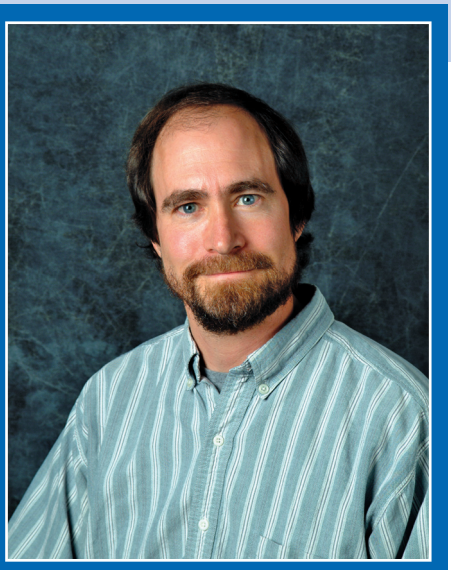
Selected Publications:

1. Derse D, Hill SA, Princler G, Lloyd P, Heidecker G. Resistance of human T cell leukemia virus type 1 to APOBEC3G restriction is mediated by elements in nucleocapsid. *Proc. Nat. Acad. Sci. USA* 104: 2915-2920, 2007.

2. Derse D, Crise B, Li Y, Princler G, Lum N, Stewart C, McGrath CF, Hughes SH, Munroe DJ, Wu X. Human T-cell leukemia virus type 1 integration target sites in the human genome: Comparison with those of other retroviruses. *J. Virol.* 81: 6731-6741, 2007.

3. Heidecker G, Lloyd PA, Soheilian F, Nagashima K, Derse D. The role of WWP1-Gag interaction and Gag ubiquitination in assembly and release of human T-cell leukemia virus type 1. *J. Virol.* 81: 9769-9777, 2007.

4. Mazurov D, Heidecker G, Derse D. The inner loop of tetraspanins CD82 and CD81 mediates interactions with HTLV-1 Gag protein. *J. Biol. Chem.* 282: 3896-3903, 2007.



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Research Interests:

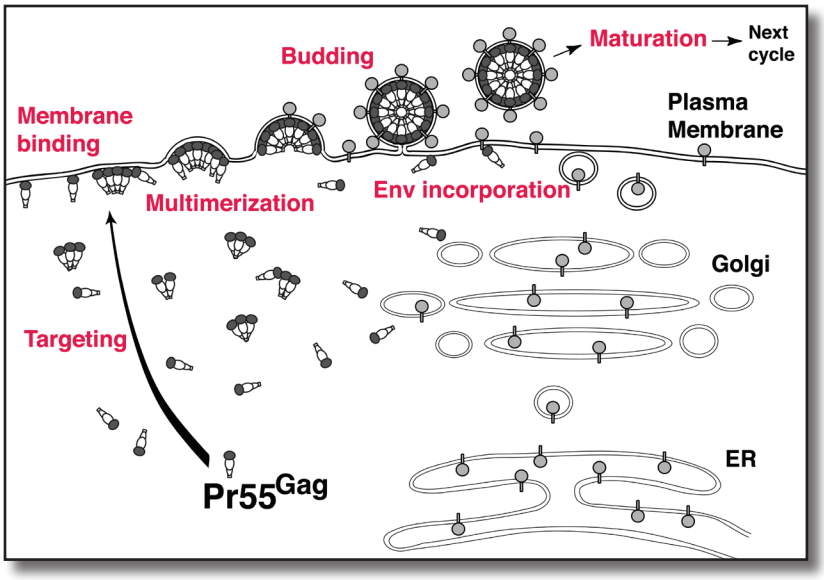
- HIV and retroviral assembly and release
- Interactions between viral and cellular proteins
- Trafficking of retroviral proteins
- Envelope glycoprotein incorporation and function

Eric O. Freed, Ph.D.

Head, Virus-Cell Interaction Section, HIV Drug Resistance Program

Biography:

Dr. Eric Freed received his Ph.D. in 1990 in the laboratories of Drs. Rex Risser and Howard Temin at the University of Wisconsin-Madison and did postdoctoral work with Dr. Temin at UW-Madison in 1991. His work in Madison focused on the function of the murine leukemia virus and HIV envelope glycoproteins in membrane fusion and virus entry. He joined the Laboratory of Molecular Microbiology at the National Institute of Allergy and Infectious Diseases (LMM/NIAID) in 1992, where he worked with Dr. Malcolm Martin on a variety of topics relating to virus assembly and entry/post-entry events in the HIV replication cycle. In 1997 he was appointed as a Tenure-Track Investigator in LMM/NIAID, and he was promoted to a tenured Senior Investigator position in 2002. Dr. Freed joined the National Cancer Institute in 2003 as Head of the Virus-Cell Interaction Section in the HIV Drug Resistance Program. Since 2004, he has served as an adjunct Associate Professor in the Department of Cell Biology and Molecular Genetics at the University of Maryland, College Park and a member of the University of Maryland Virology Program. Dr. Freed was an Organizer of the 2004 Cold Spring Harbor Retroviruses conference and the 2006 ASCB Cell Biology of Retroviruses conference. In 2009 he was appointed as the first Editor-in-Chief of *Viruses*; he also currently serves on the Editorial Boards of the *Journal of Virology, Virology, Open Virology Journal, Retrovirology,* and *Advances in Virology*.



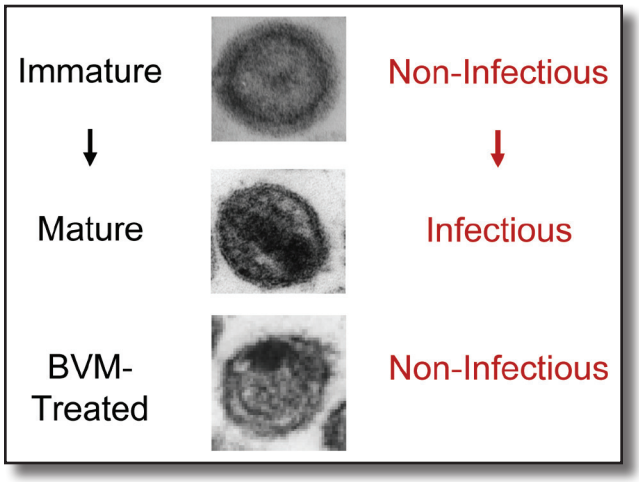
HIV particle assembly and release. Research in the Freed lab focuses primarily on the late stages of the HIV/retroviral replication cycle, in particular on those steps highlighted in red.

Research Summary:

The overall goal of the Virus-Cell Interaction Section is to understand basic mechanisms of retroviral replication at the molecular level, with an emphasis on the late stages of the HIV-1 replication cycle. Specifically, much of our current effort is aimed at understanding HIV-1 Gag trafficking, Env incorporation, virus assembly, budding, release, and maturation.

We have a special interest in the complex relationship between viral proteins and cellular factors and pathways. We believe that the elucidation of fundamental aspects of the retrovirus replication cycle will suggest novel targets for the development of antiretroviral therapies. This concept finds validation in our recent work on the HIV-1 maturation inhibitor PA-457 (bevirimat), which is currently undergoing phase IIb clinical trials in HIV-1-infected patients.

HIV-1 virions are highly enriched in cholesterol relative to the host cell plasma membrane, and multiple lines of evidence suggest that HIV-1 assembly and entry occur in cholesterol-rich lipid raft microdomains. We recently demonstrated that the cholesterol-binding compound amphotericin B methyl ester (AME) potently blocks HIV-1 entry. The passaging of HIV-1 in the presence of AME led to viral escape from the compound and mutations conferring resistance mapped to the cytoplasmic tail of gp41. Remarkably, we found that the AME-resistance mutations induce the cleavage of the gp41 cytoplasmic tail by the viral protease, leading to AME resistance. These findings reveal a novel mechanism of viral resistance to an HIV-1 entry inhibitor and constitute the first example in which HIV-1 envelope glycoprotein function is activated by the viral protease.



Blocking CA-SP1 cleavage with bevirimat (BVM) inhibits virion maturation and infectivity. Treatment with the HIV-1 maturation inhibitor PA-457 (BVM) results in a defect in Gag processing that impairs normal core condensation and blocks virus replication.

Much effort in our lab has been directed at understanding the mechanisms and itineraries of Gag trafficking in HIV-1-infected cells and the host cell machinery involved in virus budding. We previously reported that the phospholipid PI(4,5)P_2 is a key cellular cofactor in directing Gag to the plasma membrane. More recently, we found that two cellular protein families, the Golgi-localized, gamma-ear-containing, Arf-binding (GGA) and ADP ribosylation factor (Arf) proteins, play important roles in retroviral Gag trafficking and in modulating the release of virions from the cell surface. We have also recently established an imaging system that enables us to visualize Gag movement in living cells in the context of a fully infectious HIV-1 molecular clone. By using this system, we have observed that Gag moves rapidly from an internal site to the cell-cell junction, or synapse, formed between infected macrophages and uninfected T cells. These results suggest that HIV-1 virions are sequestered internally in infected macrophages but move to the synapse formed with susceptible target cells, allowing virus transmission to occur.

Selected Publications:

1. Adamson CS, Ablan SD, Boeras I, Goila-Gaur R, Soheilian F, Nagashima K, Li F, Salzwedel K, Sakalian M, Wild CT, Freed EO. In vitro resistance to the human immunodeficiency virus type 1 maturation inhibitor PA-457 (bevirimat). *J. Virol.* 80: 10957-10971, 2006.

2. Waheed AA, Ablan SD, Roser JD, Sowder RC, Schaffner CP, Chertova E, Freed EO. HIV-1 escape from the entry-inhibiting effects of a cholesterol-binding compound via cleavage of gp41 by the viral protease. *Proc. Natl. Acad. Sci. USA* 104: 8467-8471, 2007.

3. Gousset K, Ablan SD, Coren LV, Ono A, Soheilian F, Nagashima K, Ott DE, Freed EO. Real-time visualization of HIV-1 Gag trafficking in infected macrophages. *PLoS Pathog.* 4: e1000015, 2008.

4. Joshi A, Garg H, Nagashima K, Bonifacino JS, Freed EO. GGA and Arf proteins modulate retrovirus assembly and release. *Mol. Cell* 30: 227-238, 2008.

Wei-Shau Hu, Ph.D.

Head, Viral Recombination Section, HIV Drug Resistance Program

Biography:

Dr. Wei-Shau Hu received her Ph.D. in Genetics from the University of California, Davis, in 1987. She studied the mechanisms of DNA recombination that lead to human α-thalassemia in Dr. James Shen's laboratory. Under Dr. Howard Temin's guidance, she studied the mechanisms of retroviral recombination as a Postdoctoral Fellow at the University of Wisconsin. In 1991, Dr. Hu joined the faculty of West Virginia University as an Assistant Professor in the Department of Microbiology and Immunobiology and the Mary Babb Randolph Cancer Center. She was promoted to Associate Professor with tenure in 1998. In 1999, she joined the National Cancer Institute as Senior Investigator and Head of the Viral Recombination Section in the HIV Drug Resistance Program, Center for Cancer Research. Dr. Hu is an Organizer of the 2009 Cold Spring Harbor Retroviruses conference. She is also an Adjunct Professor at West Virginia University and serves on the Special Review Panel and Study Section of the National Institutes of Health extramural grant funding programs.

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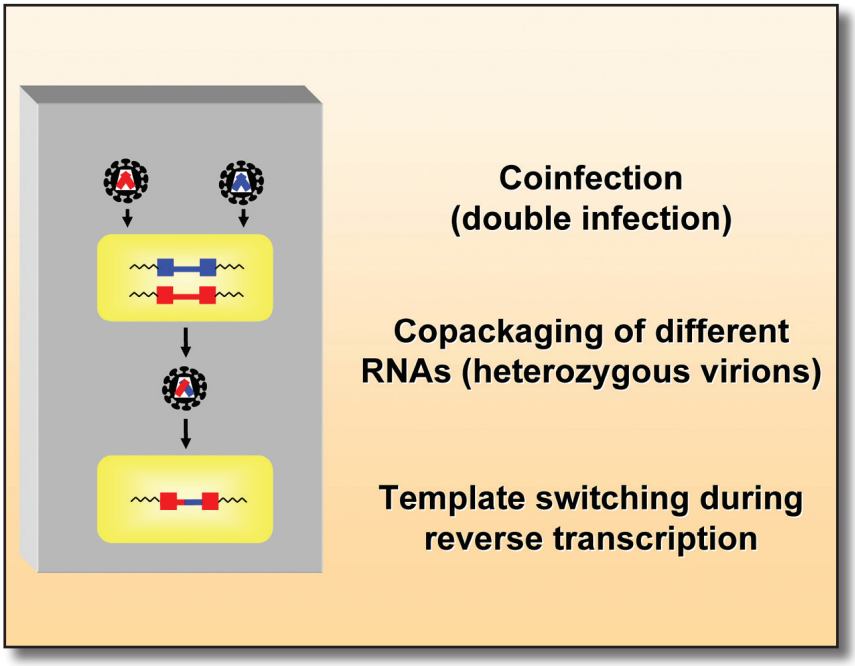
Research Interests:

- Mechanisms of reverse transcription and recombination
- RNA packaging and virus assembly
- HIV replication
- Interactions between distinct viruses



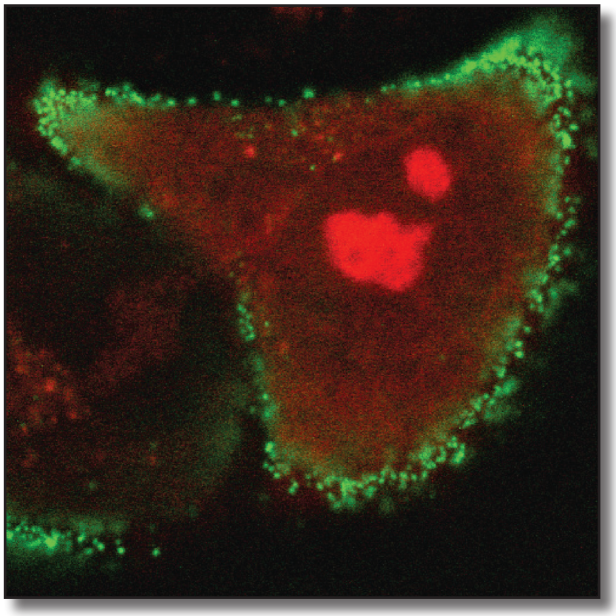
Multiple steps in viral replication can affect HIV-1 recombination. We study the mechanisms and factors that affect HIV recombination, including coinfection, heterozygous virion formation, and template-switching frequencies.

Research Summary:

We study the interactions among similar and different species of retroviruses — specifically, recombination and complementation via pseudotyping. Additionally, we study three aspects of viral infection that affect these interactions: infection by more than one virus (double infection); viral RNA packaging, including copackaging of two different RNAs (heterozygous virus); and virus assembly. Through these studies, our goal is to further our understanding of the plasticity of the retroviral genome and its replication mechanisms.

Recombination plays an important role in HIV replication and evolution. For example, many of the primary isolates of HIV are recombinants, and recombination can assort mutations to generate variants that escape host immune responses or are resistant to multidrug treatment protocols. Recombination of HIV-1 occurs during reverse transcription using information in the two RNAs to produce a hybrid DNA. Therefore, phenotypically different recombinants are generated from viruses containing two copies of different RNAs. Only cells that are doubly infected can produce virions containing two different RNAs. To better understand interactions in HIV-1 populations, we study the dynamics of double infection and the frequency of recombination.

In addition to recombination, retroviruses can interact with each other through complementation. Proteins and RNAs from two different viruses can interact in doubly infected cells. An estimated one million people are dually infected with HIV-1 and HIV-2, providing the basis for possible interactions between these two viruses in human populations. Although HIV-1/HIV-2 recombinants have not been reported thus far, given the large dual-infected human population and prior examples of novel lentiviruses generated by interspecies recombination, we cannot ignore the potential for the emergence of a hybrid virus. Our specific research aims are to investigate HIV-1 recombination and to explore genetic interactions between HIV-1 and HIV-2. In each aim, we are using experimental system studies to elucidate the mechanisms of recombination and will apply our knowledge to study patient samples. With this combined approach, we will elucidate the extent of HIV genetic recombination, thereby developing strategies to better control HIV-1 infection.



Functional complementation between proteins from different retroviruses. We study coassembly and complementation between viral proteins, including those of HIV-1 and HIV-2 Gag polyproteins. The figure shows interactions between HIV-1 and HIV-2 Gag detected by the bimolecular functional complementation assay.

Selected Publications:

1. Boyko V, Leavitt M, Gorelick R, Fu W, Nikolaitchik O, Pathak VK, Nagashima K, Hu W-S. Coassembly and complementation of Gag proteins from HIV-1 and HIV-2, two distinct human pathogens. *Mol. Cell* 23: 281-287, 2006.

2. Chen J, Pathak VK, Peng W, Hu W-S. Capsid proteins from human immunodeficiency virus type 1 and simian immunodeficiency virus SIV_{mac} can coassemble into mature cores of infectious viruses. *J. Virol.* 82: 8253-8261, 2008.

3. Motomura K, Chen J, Hu W-S. Genetic recombination between human immunodeficiency virus type 1 (HIV-1) and HIV-2, two distinct human lentiviruses. *J. Virol.* 82: 1923-1933, 2008.

4. Chin MP, Lee S-K, Chen J, Nikolaitchik OA, Powell DA, Fivash MJ Jr, Hu W-S. Long-range recombination gradient between HIV-1 subtypes B and C variants caused by sequence differences in the dimerization initiation signal region. *J. Mol. Biol.* 377: 1324-1333, 2008.

Stephen H. Hughes, Ph.D.

Director, HIV Drug Resistance Program; Chief, Retroviral Replication Laboratory; and Head, Vector Design and Replication Section, HIV Drug Resistance Program

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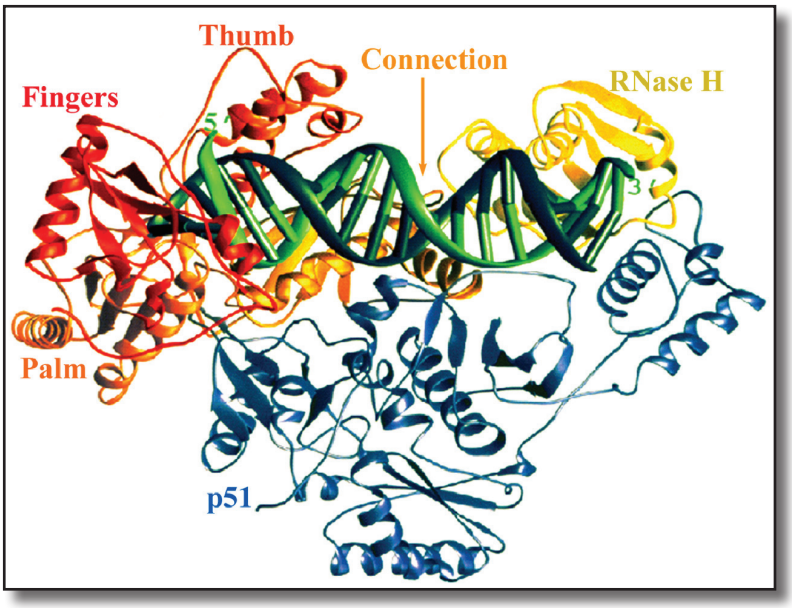
Research Interests:

- **Retroviral replication**
- **Structure and function of HIV reverse transcriptase**
- **Retroviral vectors**

Biography:

Dr. Stephen H. Hughes received his Ph.D. from Harvard University under the direction of Dr. Mario Capecchi and did postdoctoral research under the direction of Drs. J. Michael Bishop and Harold Varmus at the University of California, San Francisco. From 1979 until 1984, Dr. Hughes was a Senior Staff Investigator at Cold Spring Harbor Laboratory. In 1984, he established the Gene Expression in Eukaryotes Section in the ABL–Basic Research Program at NCI-Frederick. Dr. Hughes became Deputy Director of the ABL–Basic Research Program in 1988 and Director of the Molecular Basis of Carcinogenesis Laboratory in 1995. In 1999, he joined the HIV Drug Resistance Program in the National Cancer Institute as Chief of the Retroviral Replication Laboratory. Dr. Hughes was appointed Director of the HIV Drug Resistance Program in 2006. He is an Organizer of the Annual Symposium on Antiviral Drug Resistance and has served as an Organizer of the Retroviruses and Viral Vectors Meetings at Cold Spring Harbor Laboratory and the Annual Meeting on Oncogenes. Dr. Hughes was named one of the most frequently cited AIDS researchers by *Science Watch* in 1996.

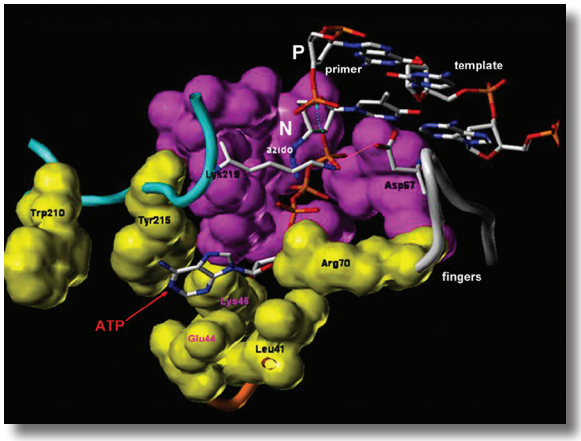


The figure shows a ribbon diagram of the structure of HIV-1 reverse transcriptase (RT) in a complex with DNA. We are engaged in a long-term study of HIV-1 RT, with the expectation that this information will be useful in developing more effective anti-RT drugs. Our strategy involves the analysis of the structure and function of both wild-type and mutant HIV-1 RTs, including drug-resistant mutants.

Research Summary:

HIV-1 is the causative agent of AIDS. Three viral enzymes — integrase, protease, and reverse transcriptase (RT) — have essential roles in the replication of HIV-1. We are engaged in a long-term effort to study HIV-1 RT, with the expectation that this information will be useful in the development of more effective anti-RT drugs. There are two classes of inhibitors of HIV-1 RT: nucleoside analogs (NRTIs) and nonnucleoside inhibitors (NNRTIs). While both have been used to successfully treat HIV-1 infections, there are serious problems with drug toxicity as well as the development of resistance.

A major focus of our work on HIV-1 RT is the mechanism(s) of RT inhibitor resistance. Our strategy has involved the biochemical and structural analysis of both wild-type and mutant HIV-1 RTs, including drug-resistant mutants. The structural studies are done by our long-term collaborator, Dr. Eddy Arnold (Rutgers University). A part of our collaboration with Dr. Arnold is intended to develop more effective NNRTIs. We are also collaborating with Dr. Victor Marquez (Laboratory of Medicinal Chemistry, NCI) to develop nucleoside analogs that are effective against NRTI-resistant viruses. Drug resistance is only one aspect of the behavior of RT. Our goals are to understand in detail how RT carries out reverse transcription in an infected cell and to correlate the wealth of structural and biochemical data on HIV-1 RT with the actual process of reverse transcription.



Model showing the binding of ATP to AZT-resistant HIV-1 RT. The basis of the most common form of AZT resistance is ATP-mediated excision of AZTMP after it has been incorporated into viral DNA by RT. The mutations that give rise to AZT resistance help ATP bind appropriately for the excision reaction. Van der Waals surfaces are drawn for polymerase active site residues (magenta) and residues involved in ATP binding and AZT resistance (yellow). Mutated amino acids M41L, K70R, L210W, and T215Y are shown with black labels, and amino acids that could be involved with ATP binding but are not mutated (E44, K46) are shown with magenta labels. The wild-type amino acids at K219 and D67 were retained in the figure to show a potential salt bridge between the residues. The AZT resistance mutations at these residues will destroy this salt bridge and may increase the ability of the pyrophosphate donor to bind. K219 and D67 are shown as stick diagrams to avoid obscuring the pyrophosphate binding site.

For this reason, we perform complementary experiments with HIV-1-based vectors in cultured cells. We have a long-term commitment to developing and using retroviral vectors, which started with the development of avian retrovirus-based RCAS vectors. We are still using the RCAS system to study viral replication. However, given the importance of HIV-1 in human disease and the wealth of information about the structure and function of the HIV-1 proteins (including RT), we particularly want to understand HIV-1 replication.

We have developed HIV-1-based vectors that carry most of the primary mutations that confer resistance to NRTIs and NNRTIs. These HIV-1-based vectors are used to study drug resistance and to evaluate the effectiveness of new anti-HIV-1 compounds. The HIV-1 vectors are also used to study important steps in the HIV 1 life cycle and to determine the effects of mutations on viral replication.

Selected Publications:

1. Oh J, Chang KW, Hughes SH. Mutations in the U5 sequences adjacent to the primer binding site do not affect tRNA cleavage by Rous sarcoma virus RNase H but do cause aberrant integrations in vivo. *J. Virol.* 80: 451-459, 2006.

2. Boyer PL, Sarafianos SG, Arnold E, Hughes SH. Why do HIV-1 and HIV-2 use different pathways to develop AZT resistance? *PLoS Pathog.* 2: e10, 2006.

3. Boyer PL, Julias JG, Ambrose Z, Siddiqui MA, Marquez VE, Hughes SH. The nucleoside analogs 4'C-methyl thymidine and 4'C-ethyl thymidine block DNA synthesis by wild-type HIV-1 RT and excision proficient NRTI resistant RT variants. *J. Mol. Biol.* 371: 873-882, 2007.

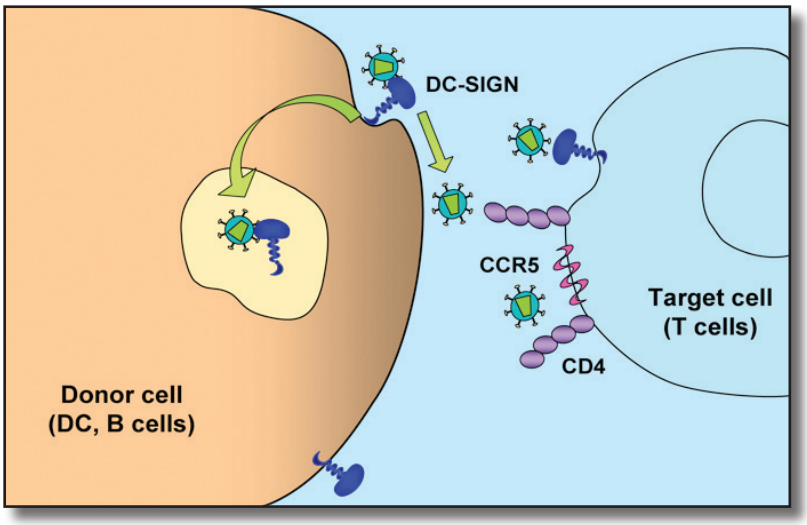
4. Das K, Bauman JD, Clark AD Jr, Frenkel YV, Lewi PJ, Shatkin AJ, Hughes SH, Arnold E. High-resolution structures of HIV-1 reverse transcriptase/TMC278 complexes: Strategic flexibility explains potency against resistance mutations. *Proc. Natl. Acad. Sci. USA* 105: 1466-1471, 2008.

Vineet N. KewalRamani, Ph.D.

Head, Model Development Section, HIV Drug Resistance Program

Biography:

Dr. Vineet N. KewalRamani first developed an interest in retroviruses as an undergraduate in the lab of Dr. Antonito T. Panganiban at the McArdle Cancer Center, University of Wisconsin at Madison. After choosing to pursue molecular biology as a career, he moved west to the University of Washington in Seattle and did his graduate work studying HIV molecular virology in the lab of Dr. Michael Emerman at the Fred Hutchinson Cancer Research Center. After completing his Ph.D. in 1996, he moved to New York to join the lab of Dr. Dan R. Littman to better understand HIV infection within an immunological context. While working in the Littman lab, Dr. KewalRamani joined in an effort to develop a murine model for HIV replication using transgenic technologies. These studies provided the foundation for his lab's current studies of understanding the blocks to HIV replication in murine cells, identifying human cell cofactors involved in HIV replication, and translating these basic science findings to develop better cell culture and animal models for HIV infection studies. Dr. KewalRamani joined the National Cancer Institute (NCI) in 1999 as Head of the Model Development Section in the HIV Drug Resistance Program. He currently serves on the Editorial Boards of the *Journal of Virology*, *PLoS One*, and *Virology* and the NIH Foundation Norman P. Salzman Virology Award and Symposium Committee and is Co-Chair of the Tenure-Track Investigator Community in the Center for Cancer Research, NCI.



DC-SIGN captures HIV through interactions with virion Env trimers. HIV complexed by DC-SIGN is efficiently released to CD4+ target cells resulting in their infection. We are mapping the contacts that HIV Env makes with DC-SIGN for initial capture and examining the mechanisms that underlie subsequent release and transmission of captured virus. Dendritic cells present at mucosal surfaces, macrophage subpopulations, and activated B cells express DC-SIGN in vivo.

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Research Interests:

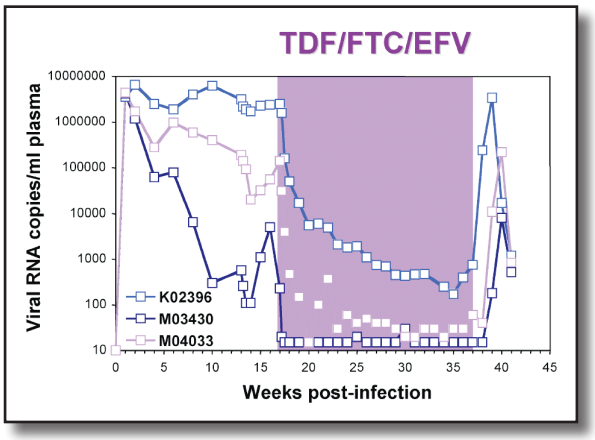
- **Virus-host interactions**
- **Cofactors in HIV infection**
- **Development of animal models for HIV infection**

Research Summary:

We have taken a two-tiered approach to advance knowledge of HIV replication and pathogenesis. The first tier is rooted in the basic science of HIV-1 replication; the second is translational and applies basic science knowledge within animal models for HIV-1 infection to ask questions only possible within these systems.

At a primary level, we are interested in defining the role of cellular factors in the transmission and early replication of HIV-1. The interaction of HIV-1 with the cell soon after infection is poorly defined. Understanding the role of HIV-1 cofactors will aid in the development of new antiviral therapies to combat HIV-1 and improved animal models in which to study HIV-1 replication. Projects in this area include identifying host factors that aid in HIV-1 uncoating after entry, defining the pathway of HIV-1 trafficking from cytoplasm to nucleus in nondividing cells, and examining the mechanisms through which the extracellular factor DC-SIGN captures HIV-1 particles and facilitates their transfer to target cells.

Second, we have developed a new pigtail macaque model in which to study the evolution of antiviral resistance and examine the presence of persisting reservoirs during antiretroviral therapy (ART). While pigtail macaques are not susceptible to infection with HIV-1, simian immunodeficiency virus (SIV), a related virus, efficiently infects these animals and causes an immunodeficiency and pathogenesis that are characteristic of AIDS. Because of genetic differences, SIV is not responsive to many drugs used to treat HIV-1. We therefore spliced a portion of HIV-1 in the SIV genome to create a hybrid virus referred to as RT-SHIV. We showed that RT-SHIV infection of pigtail macaques leads to immune cell depletion and pathogenesis. Importantly, we also demonstrated that a drug cocktail commonly used to treat HIV-1-infected patients was highly effective in suppressing RT-SHIV in the pigtail macaques and preventing immune cell depletion. Furthermore, we observed that animals with prior transient exposure to only one drug from the cocktail developed antiviral resistance more quickly when treated with the more potent three-drug combination. In animals that did not develop resistance, virus rebounded to pretherapy levels when ART was stopped, indicating persisting reservoirs of infected cells.



Potent suppression of RT-SHIV during tenofovir (TDF), emtricitabine (FTC), and efavirenz (EFV) combination therapy, a common regimen for HIV-infected patients. RT-SHIV is derived from a pathogenic SIVmne isolate and encodes HIV-1 RT. EFV, a nonnucleoside HIV-1 RT inhibitor, is otherwise ineffective against SIV RT molecules.

While our current infection model leverages a modified SIV encoding HIV-1 RT, infection models under development with intra- and extramural collaborators rely on HIV-1 containing minimal portions of SIV to increase tropism and virulence in macaques. Infection of macaques with closer facsimiles of HIV-1 will permit the use of powerful antiviral drug combinations to seek tissues where virus is able to persist and escape eradication.

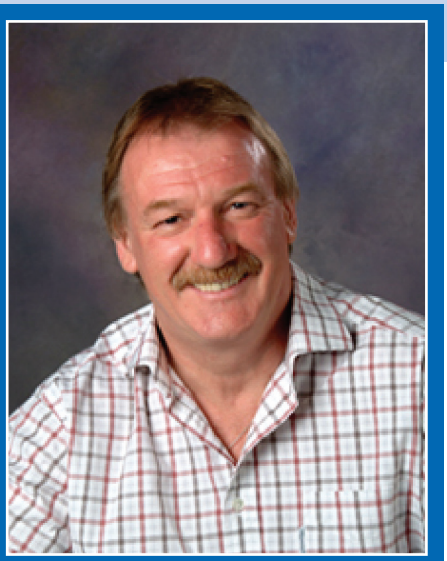
Selected Publications:

1. Lee K, KewalRamani VN. In defense of the cell: TRIM5α interception of mammalian retroviruses. *Proc. Natl. Acad. Sci. USA* 101: 10496-10497, 2004.

2. Ambrose Z, Palmer S, Boltz VF, Kearney M, Larsen K, Polacino P, Flanary L, Oswald K, Piatak M, Smedley J, Shao W, Bischofberger N, Maldarelli F, Kimata JT, Mellors JW, Hu SL, Coffin JM, Lifson JD, KewalRamani VN. Suppression of viremia and evolution of human immunodeficiency virus type 1 drug resistance in a macaque model for antiretroviral therapy. *J. Virol.* 81: 12145-12155, 2007.

3. Ambrose Z, Compton L, Piatak M Jr, Lu D, Alvord WG, Lubomirski MS, Hildreth JEK, Lifson JD, Miller CJ, KewalRamani VN. Incomplete protection against simian immunodeficiency virus vaginal transmission in rhesus macaques by a topical antiviral agent revealed by repeat challenges. *J. Virol.* 82: 6591-6599, 2008.

4. Hatziioannou T, Ambrose Z, Chung NPY, Piatak M Jr, Yuan F, Trubey CM, Coalter V, Kiser R, Schneider D, Smedley J, Pung R, Gathuka M, Estes JD, Veazey RS, KewalRamani VN, Lifson JD, Bieniasz PD. A macaque model of HIV-1 infection. *Proc. Natl. Acad. Sci. USA* 106: 4425-4429, 2009.

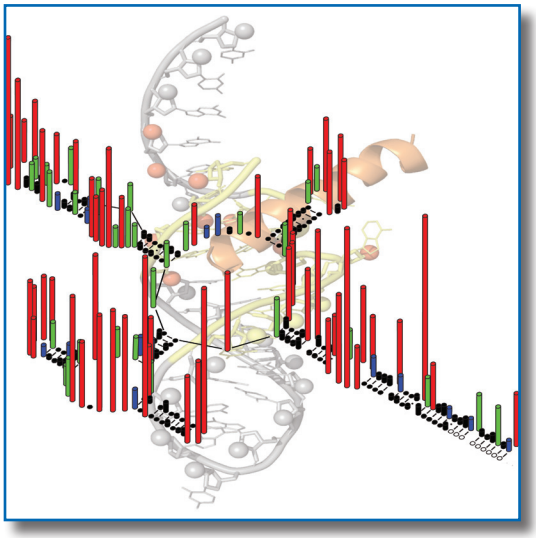


Stuart F.J. Le Grice, Ph.D.

Head, Center of Excellence in HIV/AIDS & Cancer Virology, Center for Cancer Research; and Head, RT Biochemistry Section, HIV Drug Resistance Program

Biography:

Dr. Stuart Le Grice received his Ph.D. from the Department of Biochemistry, University of Manchester, UK, in 1976, studying mechanisms of R-factor-mediated multi-drug resistance in *Escherichia coli*. After postdoctoral training in the United Kingdom, Germany, and the United States, he was appointed Senior Scientist in the Central Research Units of Hoffmann La Roche, Switzerland, where he worked from 1984 to 1990 evaluating HIV-1 and HIV-2 enzymes as therapeutic targets. In 1990, he was recruited to the Division of Infectious Diseases, Department of Medicine, Case Western Reserve University (CWRU), Cleveland, OH, receiving tenure in 1992, and promotion in 1995 to Professor of Medicine, Biochemistry, and Oncology. From 1994 to 1999, he directed the NIH-accredited CWRU Center for AIDS Research. Dr. Le Grice joined the National Cancer Institute in 1999 as Chief of the Resistance Mechanisms Laboratory in the HIV Drug Resistance Program, Center for Cancer Research (CCR). In 2006, he was appointed Head of the Center of Excellence in HIV/AIDS & Cancer Virology, CCR and is also a Steering Committee Member of the Molecular Targets Discovery Program and the Chemistry and Structural Biology Faculty. In addition to serving on the Editorial Board of the *Journal of Biological Chemistry*, Dr. Le Grice has been both an *ad hoc* and permanent Study Section member of several NIH AIDS review panels, as well as an *ad hoc* reviewer for multiple international funding agencies. In 2007, Dr. Le Grice was designated an NCI Mentor of Merit.



Examining the 2-D structure of inhibitor-resistant variants of the HIV-1 Rev response element by selective 2'-hydroxyl acylation analyzed by primer extension (SHAPE).

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Research Interests:

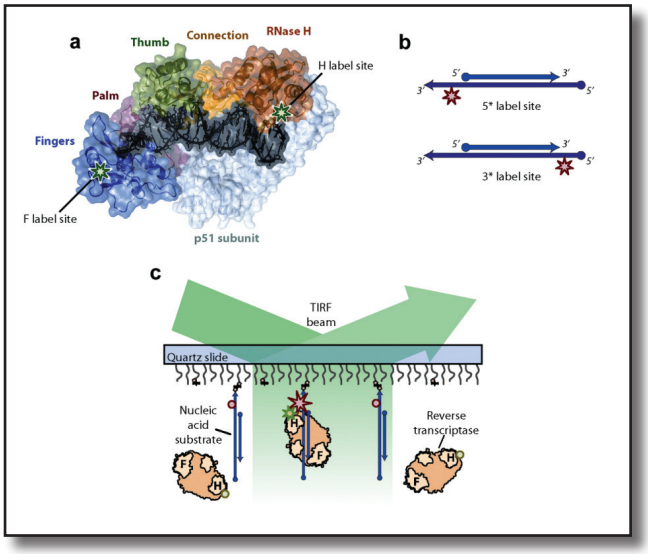
- **Protein and nucleic acid footprinting**
- **Bioconjugate evaluation of reverse transcription complexes**
- **RNase H inhibitors**
- **Unnatural amino acids as probes of protein function**

Research Summary:

Novel drugs targeting virus entry and maturation have recently been added to the growing armory of antiviral agents aimed at stemming the continuing spread of HIV infection. In addition, host factors participating in key steps in HIV replication are under evaluation as targets for therapeutic intervention. Despite these promising approaches, the viral enzymes protease (PR), reverse transcriptase (RT), and integrase (IN) remain the primary targets of combination antiretroviral therapy, with more than 20 drugs in clinical use against these enzymes.

Research in the RT Biochemistry Section (RTBS) combines biochemistry and biophysics with structural, molecular, and chemical biology to examine, at the molecular level, the manner in which retroviral RT and its counterpart from *Saccharomyces cerevisiae* retrotransposons convert the single-stranded RNA genome into integration-competent double-stranded DNA.

Nucleoside analogs imparting different properties into nucleic acid duplexes (eliminating hydrogen bonding or base stacking, decreasing duplex flexibility, neutralizing the phosphate backbone) are exploited to provide novel acumen into the interaction of the retroviral polymerase with *cis*-acting regulatory elements.



Probing the orientational dynamics of HIV-1 RT on duplex and hybrid nucleic acid substrates via single-molecule spectroscopy. The fluorophores Cy3 and Cy5 are introduced at different sites of HIV-1 RT (a) and the nucleic acid substrate (b), respectively. The intensity of the FRET signal (c) is directly related to the proximity of Cy3 and Cy5. A detailed explanation of the experimental setup and use in determining enzyme orientation is given in Reference 2.

Nucleic acid strategies are complemented by the use of genetically engineered *Escherichia coli* to site-specifically introduce unnatural amino acids with a variety of biophysical properties into recombinant proteins. This approach has created novel HIV-1 RT mutants conferring resistance to inhibitors of both DNA polymerase and ribonuclease H (RNase H) function.

Chemical footprinting of RNA by automated selective 2′-hydroxyl acylation analyzed by primer extension (SHAPE) is under development to examine the structure of regulatory elements on the viral RNA genome both *in vitro* and *in vivo*, while high-resolution FTICR-ESI mass spectrometry has been combined with NMR spectroscopy to examine the interaction of small-molecule ligands with the polypurine tract primer of plus-strand DNA synthesis.

Finally, we have initiated single-molecule fluorescence studies to probe the orientation of RT on a variety of nucleic acid duplexes. Knowledge gained through these multidisciplinary approaches helps characterize potent and selective RNase H inhibitors identified by high-throughput screening. The RTBS enjoys the expertise of investigators and resources within the Intramural Research Program while collaborating with internationally recognized extramural investigators to promote new areas of investigation.

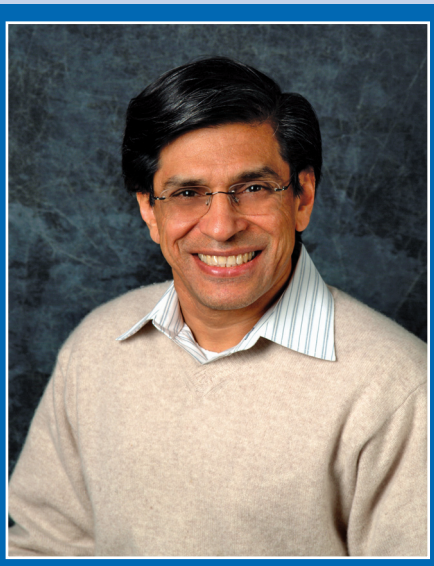
Selected Publications:

1. Legiewicz M, Badorrek CS, Turner KB, Fabris D, Hamm TE, Rekosh D, Hammarskjöld M-L, Le Grice SFJ. Resistance to RevM10 inhibition reflects a conformational switch in the HIV-1 Rev response element. *Proc. Natl. Acad. Sci. USA* 105: 14365-14370, 2008.

2. Abbondanzieri EA, Bokinsky G, Rausch JW, Zhang J, Le Grice SFJ, Zhuang X. Dynamic binding orientations direct activity of HIV reverse transcriptase. *Nature* 453: 184-189, 2008.

3. Wendeler M, Lee H-F, Bermingham A, Miller JT, Chertov O, Bona MK, Baichoo NS, Ehteshami M, Beutler J, O'Keefe BR, Götte M, Kvaratskhelia M, Le Grice SFJ. Vinylogous ureas as a novel class of inhibitors of reverse transcriptase-associated ribonuclease H activity. *ACS Chem. Biol.* 3: 635-644, 2008.

4. Liu S, Abbondanzieri EA, Rausch JW, Le Grice SFJ, Zhuang X. Slide into action: Dynamic shuttling of HIV reverse transcriptase on nucleic acid substrates. *Science* 322: 1092-1097, 2008.



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Research Interests:

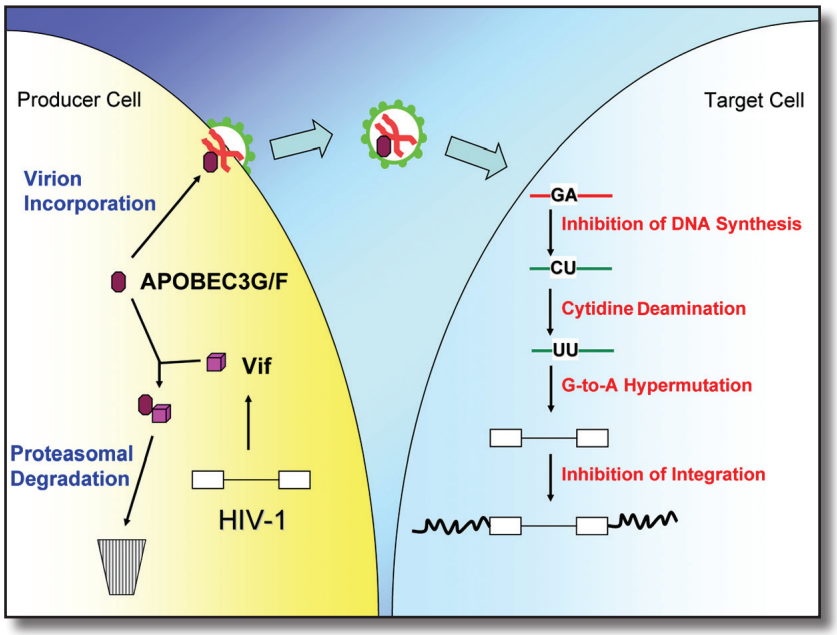
- **Structure and function of APOBEC3 host restriction factors**
- **Mechanisms of antiviral drug resistance**
- **Reverse transcriptase structure, fidelity, and template switching**

Vinay K. Pathak, Ph.D.

Head, Viral Mutation Section, HIV Drug Resistance Program

Biography:

Dr. Vinay K. Pathak received his B.A. in Biology from the University of California, Los Angeles, in 1979, and his M.S. in Comparative Pathology from the University of California, Davis (UCD), in 1983 for characterization of mouse mammary tumor virus proviral integration sites near the *int1* and *int2* loci in Dr. Robert Cardiff's laboratory. He received his Ph.D. in Genetics for work on characterization of the eukaryotic protein synthesis initiation factors eIF-2α and eIF-2β in Dr. John W.B. Hershey's laboratory (UCD) in 1988. From 1988 to 1991, he was a Postdoctoral Fellow under the guidance of Dr. Howard Temin at the University of Wisconsin, where he studied the *in vivo* fidelity and nature of mutations that arise during retroviral replication. In 1991, Dr. Pathak became an Assistant Professor in the Department of Biochemistry and the Mary Babb Randolph Cancer Center at West Virginia University. He was promoted to Associate Professor with tenure in 1998. He joined the National Cancer Institute in 1999 as Senior Investigator and Head of the Viral Mutation Section in the HIV Drug Resistance Program. Dr. Pathak was appointed as Guest Editor for the HIV Drug Resistance special issue of *Viruses*, which will be published in October 2009.



In virus producer cells, APOBEC3 proteins are incorporated into HIV-1 virions in the absence of Vif. Vif binds to APOBEC3 proteins and targets them for proteasomal degradation. APOBEC3 proteins inhibit viral DNA synthesis, induce G-to-A hypermutation, and inhibit viral DNA integration.

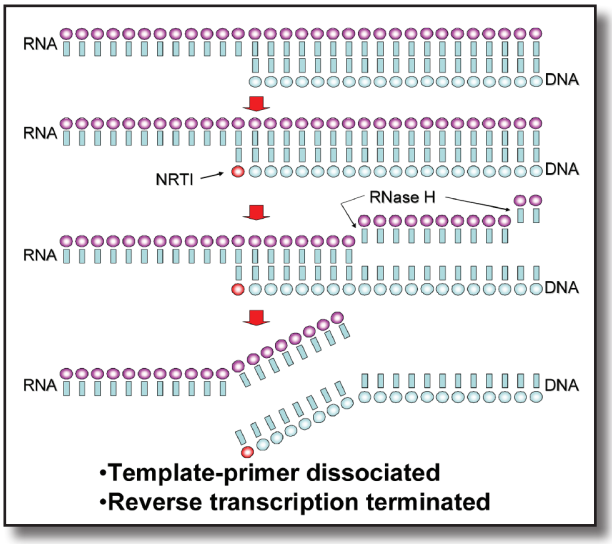
Research Summary:

Our goal is to understand how HIV-1 replicates and how its interactions with the host cell influence its replication. We are active in three major research areas. First, we are defining the structure and function of APOBEC3G and 3F proteins. We are identifying the determinants of interaction between HIV-1 Vif and APOBEC3 proteins through genetic and biochemical analysis. We are elucidating the mechanisms by which the APOBEC3 proteins inhibit HIV-1 DNA synthesis, integration, and viral replication. In addition, we are investigating the mechanisms by which APOBEC3 proteins are incorporated into virions and regulated *in vivo*. Our long-term goal is to identify small-molecule inhibitors that interfere with the interaction between HIV-1 Vif and APOBEC3 proteins, which could form the basis of a new class of antiretroviral drugs.

Second, we are elucidating mechanisms by which HIV-1 reverse transcriptase (RT) acquires resistance to RT inhibitors. We have identified eight novel mutations in the C-terminal connection and RNase H domains of RT that substantially enhance NRTI and NNRTI resistance.

Current genotypic and phenotypic studies only analyze the N-terminal domains of RT for drug resistance and do not include the C-terminal domains. However, our studies indicate that it is important to include the C-terminal connection and RNase H domains in future genotypic and phenotypic analyses of drug resistance. The improved genotypic and phenotypic assays might predict antiviral drug resistance more accurately and would lead directly to the development of new clinical protocols designed to improve drug choice in salvage therapy. Our specific research aims are to further define the role of the C-terminal connection and RNase H domains of RT in antiviral drug resistance in subtype B as well as non-B subtypes of HIV-1.

Third, we are exploring the *in vivo* mechanisms of reverse transcription by using a novel quantitative strand-specific amplification (SSA) technology that we recently developed. We are using the SSA method to measure the rates of viral DNA synthesis in activated primary CD4+ T cells and macrophages, the target cells of HIV-1 infection. We are studying the mechanism of discontinuous plus-strand DNA synthesis and the influence of RT inhibitors on the kinetics of reverse transcription, providing insights into their mechanisms of inhibition. The SSA technology provides a novel approach to analyzing DNA replication processes and may facilitate the development of new antiretroviral drugs that target specific steps in HIV-1 reverse transcription.



A mechanism by which C-terminal domain mutations increase NRTI resistance. NRTI is incorporated into DNA during RNA-template-directed DNA synthesis; RNase H degrades template RNA; the template and primer are then dissociated, leading to termination of reverse transcription. Mutations that reduce RNase H activity provide more time for NRTI excision to occur before template RNA degradation, resulting in increased NRTI resistance.

Selected Publications:

1. Nikolenko GN, Delviks-Frankenberry KA, Palmer S, Maldarelli F, Fivash MJ Jr, Coffin JM, Pathak VK. Mutations in the connection domain of HIV-1 reverse transcriptase increase AZT resistance. *Proc. Natl. Acad. Sci. USA* 104: 317-322, 2007.

2. Mbisa JL, Barr R, Thomas JA, Vandegraaff N, Dorweiler IJ, Svarovskaia ES, Brown WL, Mansky LM, Gorelick RJ, Harris RS, Engelman A, Pathak VK. Human immunodeficiency virus type 1 cDNAs produced in the presence of APOBEC3G exhibit defects in plus-strand DNA transfer and integration. *J. Virol.* 81: 7099-7110, 2007.

3. Delviks-Frankenberry KA, Nikolenko GN, Boyer PL, Hughes SH, Coffin JM, Jere A, Pathak VK. HIV-1 reverse transcriptase connection subdomain mutations reduce template RNA degradation and enhance AZT excision. *Proc. Natl. Acad. Sci. USA* 105: 10943-10948, 2008.

4. Russell RA, Smith J, Barr R, Bhattacharyya D, Pathak VK. Distinct domains within APOBEC3G and APOBEC3F interact with separate regions of human immunodeficiency virus type 1 Vif. *J. Virol.* 83: 1992-2003, 2009.

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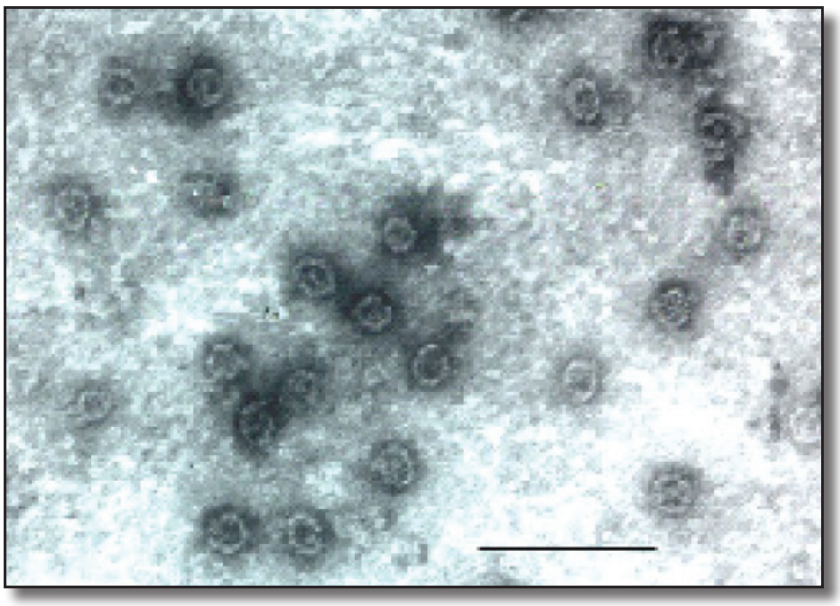
Research Interests:

- **Molecular mechanisms of retroviral assembly and replication**
- **Interactions of retroviral proteins with nucleic acids**
- **Interactions of retroviral components with host antiviral factors**

Head, Retrovirus Assembly Section, HIV Drug Resistance Program

Biography:

Dr. Alan Rein obtained his Ph.D. with Dr. Harry Rubin at the University of California at Berkeley and did postdoctoral research as an American Cancer Society Fellow under the direction of Dr. Sheldon Penman at the Massachusetts Institute of Technology. Dr. Rein has been associated with the National Cancer Institute (NCI) since 1976 and served as Head of the Retroviral Genetics Section in the ABL–Basic Research Program from 1984 to 1999. In 1999, he joined the HIV Drug Resistance Program as Head of the Retrovirus Assembly Section and was appointed to the NCI Senior Biomedical Research Service. Dr. Rein was an Organizer of the 1995 Cold Spring Harbor Laboratory Meeting on Retroviruses and the 2001 International Retroviral Nucleocapsid Symposium. He currently serves on the Editorial Boards of the *Journal of Biological Chemistry, Virology,* and *Journal of Virology*. He is also a member of the Center of Excellence in HIV/AIDS & Cancer Virology and the Chemistry and Structural Biology Faculty in the Center for Cancer Research, NCI. His research has dealt with a number of aspects of the biology and molecular biology of murine and human retroviruses, including virus assembly and maturation, viral envelope function, translational suppression, and pathogenesis.



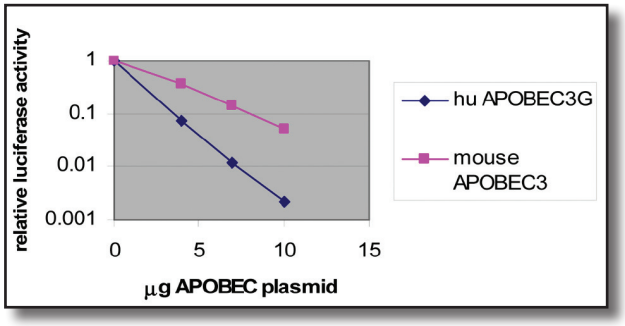
Virus-like particles assembled from purified HIV-1 Gag protein that had been produced in bacteria and mixed with E. coli *ribosomal RNA. Scale bar: 100 nm.*

Research Summary:

Virus particle assembly is an essential step in the HIV 1 replication cycle. This fact makes it a potential target for antiviral therapy. However, it has not been exploited therapeutically to date, presumably because the molecular mechanisms responsible for assembly are not understood in detail.

Our research focuses on the molecular mechanisms underlying the assembly of infectious particles of HIV-1 and other retroviruses. Expression of a single viral protein, termed Gag, is sufficient for the efficient assembly of retrovirus particles in mammalian cells. Virus assembly can be recapitulated in a fully defined system, containing only purified recombinant Gag protein and nucleic acid (Fig. 1). We have found that our recombinant Gag protein is in a folded, compact conformation in solution; in contrast, it is rod-shaped in the assembled virus particle. Therefore, our findings show for the first time that the Gag protein undergoes a major conformational change before or during virus assembly. This is a previously unsuspected event in virus replication, and represents a new potential target for antiviral therapy. Current efforts are directed toward identifying the "trigger" for this conformational change.

We have also reported that the same site within Gag functions successively in two different steps in the replication cycle: first, in the initial assembly of the virus particle, and second, in formation of the mature core following cleavage of Gag within the released particle. It is remarkable that the same site is used twice in the viral replication cycle; this site would appear to be a particularly attractive target for therapeutic intervention.



Inactivation of murine leukemia virus by mouse APOBEC3 and by human APOBEC3G. A murine leukemia virus-derived "reporter vector" encoding luciferase was produced in the presence of increasing amounts of either mouse APOBEC3 or human APOBEC3G expression vectors, and its infectivity was determined by assaying for luciferase following infection of fresh cells.

We have found that RNA plays a crucial role in retrovirus particle structure, and are studying the nature of this contribution. Gag normally selects the viral genomic RNA for packaging into the assembling particle; the mechanism of this selection is unknown. We found that if this RNA is not present in the cell, Gag packages cellular mRNA molecules; most, but not all, mRNAs are packaged nonselectively. We are now analyzing how certain RNAs are enriched in virus particles.

Another focus of our research is the interactions between retroviruses and antiviral factors produced by their hosts. Mouse cells encode an antiviral factor termed APOBEC3. We found that murine retroviruses are almost entirely resistant to its antiviral effects (Fig. 2 — compared with its human counterpart APOBEC3G). In collaboration with Dr. David Derse (HIV Drug Resistance Program), we are investigating the mechanism underlying this relative resistance of murine leukemia virus to APOBEC3, using a number of experimental approaches. These studies should help elucidate the mechanisms of encapsidation of, and virus inactivation by, APOBEC proteins.

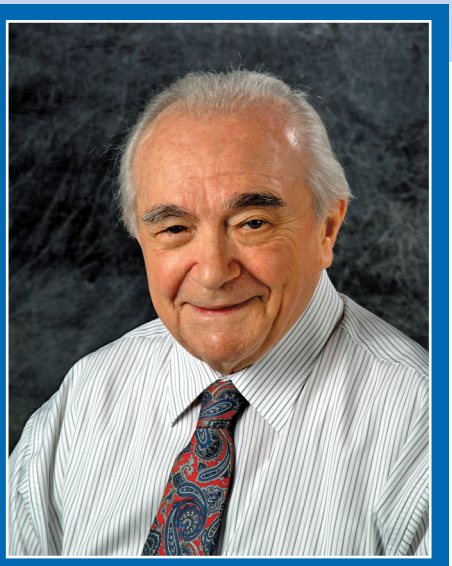
Selected Publications:

1. Fisher RJ, Fivash MJ, Stephen AG, Hagan NA, Shenoy SR, Medaglia MV, Smith LR, Worthy KM, Simpson JT, Shoemaker R, McNitt KL, Johnson DG, Hixson CV, Gorelick RJ, Fabris D, Henderson LE, Rein A. Complex interactions of HIV-1 nucleocapsid protein with oligonucleotides. *Nucleic Acids Res.* 34: 472-484, 2006.

2. Rulli SJ, Hibbert CS, Mirro J, Pederson T, Biswal S, Rein A. Selective and nonselective packaging of cellular RNAs in retrovirus particles. *J. Virol.* 81: 6623-6631, 2007.

3. Datta SA, Zhao Z, Clark PK, Tarasov S, Alexandratos JN, Campbell SJ, Kvaratskhelia M, Lebowitz J, Rein A. Interactions between HIV-1 Gag molecules in solution: An inositol phosphate-mediated switch. *J. Mol. Biol.* 365: 799-811, 2007.

4. Datta SA, Curtis JE, Ratcliff W, Clark PK, Crist RM, Lebowitz J, Krueger S, Rein A. Conformation of the HIV-1 Gag protein in solution. *J. Mol. Biol.* 365: 812-824, 2007.



Stephen Oroszlan, Ph.D.

Scientist Emeritus, HIV Drug Resistance Program

Biography:

Dr. Stephen Oroszlan obtained his Ph.D. in Pharmacology from Georgetown University. After his postdoctoral training at the National Cancer Institute (NCI) from 1961 to 1963, he conducted biochemical research on retroviruses at the Albert Einstein Medical Center, George Washington University School of Medicine, and Flow Laboratories. In 1976, he joined the Biological Carcinogenesis Program at NCI-Frederick as Head of the Immunochemistry Section, and served as Director of the Molecular Virology and Carcinogenesis Laboratory, ABL–Basic Research Program, from 1983 to 1995. Dr. Oroszlan was appointed Scientist Emeritus in 1995 and has been affiliated with the HIV Drug Resistance Program since 1999. His studies have provided invaluable information on the biosynthesis of retroviral enzymes and structural proteins. He has published more than 250 papers and was named one of the most highly cited researchers in microbiology by the Institute for Scientific Information. Dr. Oroszlan has been awarded three U.S. patents and one European patent on HIV protease and one U.S. patent on chloroquine as an inhibitor of infectious HIV production. His numerous honors include the award of a Doctor Honoris Causa Degree from the University of Debrecen Medical School in 1993, election to the Hungarian Academy of Sciences as a Foreign Associate in 1994, a Retroviral Retrospective Symposium to Honor Dr. Stephen Oroszlan held at Hood College in 1994, and the Mór Kaposi Research Foundation Award in 2000.

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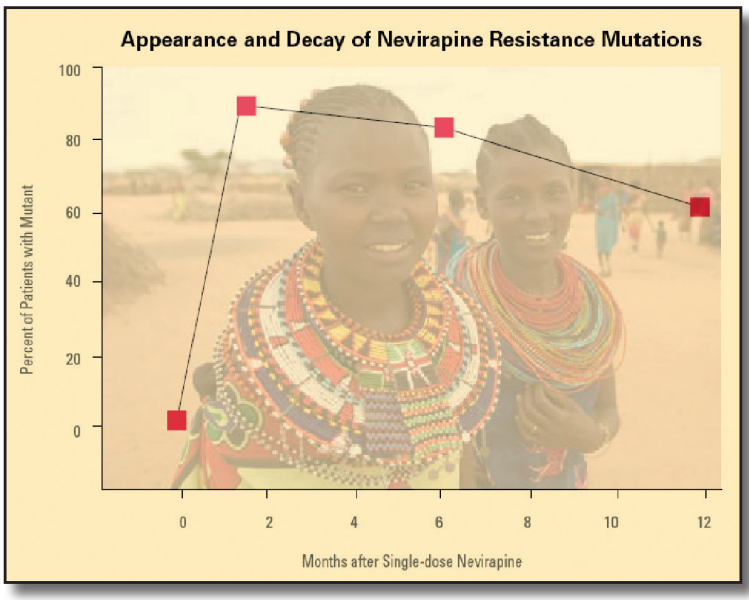
Research Interests:

- **Biochemistry and enzymology of retroviral replication**
- **Retroviral enzymes and structural proteins**
- **Structure and function of HIV protease**

At the Symposium on HIV Protease and Beyond: The Past, Present, and Future of HIV Structural Biology, held at NCI-Frederick in January 2009, Dr. Michael Gottesman (NIH Deputy Director for Intramural Research) presented an award to Dr. Stephen Oroszlan in recognition of his many contributions to our understanding of the structure of retroviruses, and for his seminal work on viral proteases. Dr. Oroszlan gave the opening talk at the symposium, describing the key events in retroviral protease research and the discovery of HIV protease.

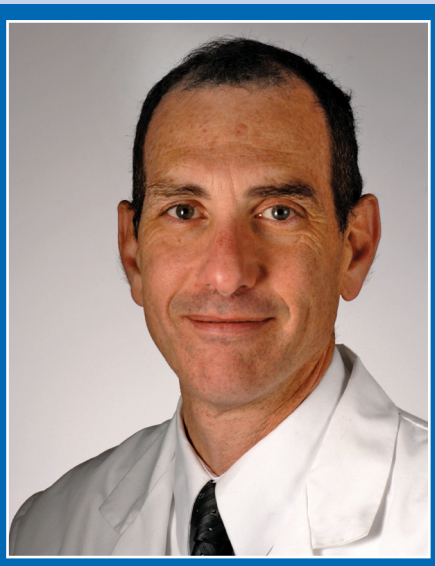
Clinical Program — Host-Virus Interaction Branch

As the clinical arm of the HIV Drug Resistance Program, the Host-Virus Interaction Branch (HVIB) conducts fundamental studies on the nature of HIV drug resistance *in vivo*. The HVIB has developed sensitive and specific tools that are being used to analyze patient material to address questions of HIV population genetics, replication dynamics, and emergence of resistance in infected individuals. Ongoing studies are focused on several issues: understanding the origin and development of genetic variation in HIV populations, and using this information to infer the structure of the underlying population of HIV-infected cells; understanding the extent of replication of HIV— and the potential for evolution of resistance — in patients whose virus load has become suppressed under successful therapy; and understanding the mechanism, kinetics, and consequences of appearance and disappearance of drug resistance mutations in infected patients before, during, and after treatment.



The HVIB comprises the following elements:

- The In Vivo Biology Group, headed by **Dr. Frank Maldarelli**, directs the protocol development, regulatory affairs, patient recruitment, and sample collection effort in the NIH Clinical Center, in collaboration with the AIDS clinical research programs of the National Cancer Institute (HIV/AIDS Malignancy Branch, Dr. Robert Yarchoan), National Institute of Allergy and Infectious Diseases (Division of Clinical Research, Dr. H. Clifford Lane), and NIH Clinical Center (Critical Care Medicine Department, Dr. Henry Masur). The In Vivo Biology Group is studying patient samples derived from its own protocols (largely in collaboration with these NIH clinical groups) and obtained from numerous extramural collaborators.
- The Virology Core, led by **Dr. Mary Kearney**, is responsible for the development and implementation of new assays to detect and analyze HIV in patient samples. These assays are principally used by the HVIB and other sections of the HIV Drug Resistance Program.
- **Dr. John Coffin**, American Cancer Society Research Professor of Molecular Biology and Microbiology at Tufts University and founding Director of the HIV Drug Resistance Program, now serves as advisor to the Director of the Center for Cancer Research and consultant to the clinical program, particularly in the areas of assay design and interpretation, study design, and data analysis.
- Collaboration with **Dr. John Mellors** from the University of Pittsburgh provides critical consultation and advice on all aspects of HIV clinical research, including protocol design and implementation. Dr. Mellors provides an invaluable interface with the extramural HIV research community, including the AIDS Clinical Trials Group and international collaborative trials, making possible the Program's participation in major multicenter trials. The Mellors laboratory also collaborates closely with the HVIB on development of novel laboratory techniques for analysis of clinical material, and in basic studies of the mechanism of resistance to reverse transcriptase inhibitors.



Frank Maldarelli, M.D., Ph.D.

Head, In Vivo Biology Group, HIV Drug Resistance Program

Biography:

Dr. Frank Maldarelli received his Ph.D. from the City University of New York and his M.D. from Mount Sinai School of Medicine. After completing his residency in internal medicine at The Columbia-Presbyterian Hospital, New York, he joined the Laboratory of Molecular Microbiology at the National Institute of Allergy and Infectious Diseases (NIAID) as a Medical Staff Fellow. Dr. Maldarelli joined the National Cancer Institute (NCI) in 1998 as Head of the In Vivo Biology Group in the HIV Drug Resistance Program (DRP). Representing the clinical research arm of the DRP, he has established extensive collaborations between the DRP in Frederick and both the NCI HIV and AIDS Malignancy Branch and the NIAID AIDS clinical research program in Bethesda. Dr. Maldarelli is an Attending Physician in the NIAID/CCMD HIV service, and is currently a faculty member of the International Society of Infectious Diseases HIV Training Program, NIH Infectious Disease Consult Service, and Center for Bio-Medical Communications Infectious Disease Board Review Course, and a lecturer in the Washington D.C. community outreach PACT Program.

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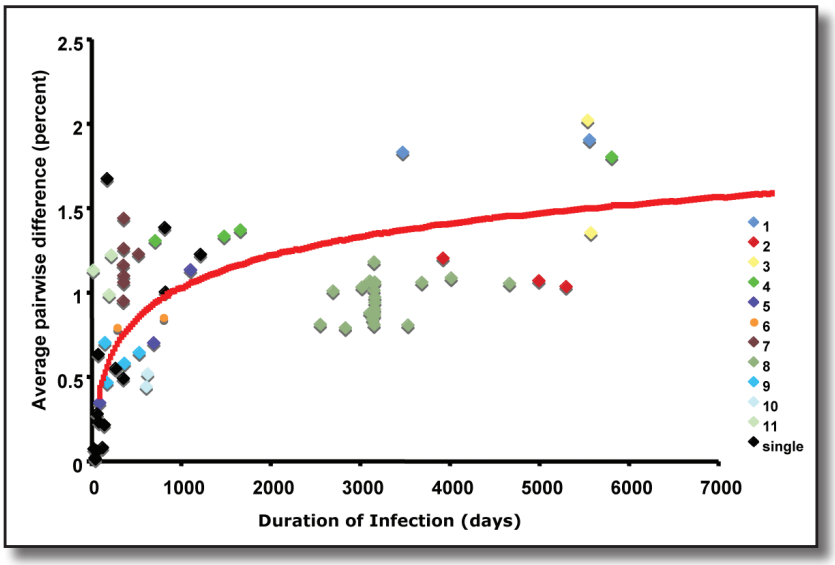
Research Interests:

- **HIV-1 population genetics**
- **Source of low-level viremia in patients suppressed on anti-retroviral therapy**
- **Clinical studies on HIV resistance**



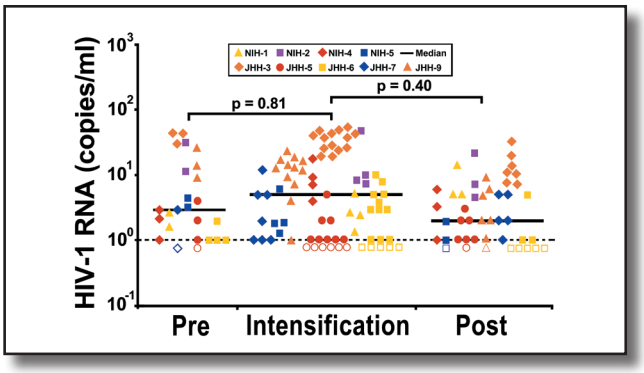
Analysis of genetic diversity of HIV-1 in patients with HIV-1 infection. HIV-1 genetic diversity (expressed as percent average pairwise difference) was determined from alignments of single-genome sequences obtained from all samples from 27 HIV-1-infected patients and presented as a function of minimum duration of infection. Individual patients with multiple samples are color coded as indicated. Patients for whom only a single sample was available for analysis are coded in black. Early in infection (~days 1-200), diversity accumulated rapidly and approximated the mutation rate of HIV-1 reverse transcriptase (RT). In established HIV-1 infection, diversity accumulates slowly, and is likely the product of a balance of mutation and selection.

Research Summary:

The In Vivo Biology Group develops and implements clinical protocols to elucidate mechanisms underlying the emergence of resistance *in vivo*, the dynamics of infection under treatment, and the role of resistance mutations in the efficacy and failure of subsequent treatments.

In the first of four projects conducted by the In Vivo Biology Group, our goal is to determine the dynamics of viral replication in HIV-infected patients on suppressive antiretroviral therapy. These studies have been extended in clinical trials performed at NIH and elsewhere to determine the levels of viremia when treatment regimens are either simplified or intensified. Initial results from these trials indicate that the source of HIV-1 viremia during suppressive antiretroviral therapy is not from active cycles of replication, but from reservoirs of long-lived cells. We are also exploring new strategies to decrease reservoirs of HIV-1 in infected individuals.

In a second research project, we are investigating the genetic structure of HIV populations in infected individuals. We are using the single-genome sequencing technology developed by the Virology Core to analyze and understand the accumulation of genetic variation in the *gag/pol* and *env* genes of HIV-1 in a number of different patient groups, including chronically infected patients, both naïve and on therapy, as well as in primary and early HIV-1 infection. The goal of this study is to understand the nature of the forces (mutation, selection, drift, recombination) that mold the genetic diversity of virus populations before and after antiretroviral therapy is introduced.



Antiretroviral therapy intensification does not reduce HIV-1 viremia. HIV-1 RNA levels are shown for all time points for all nine patients. Each set of colored symbols represents HIV-1 RNA values obtained for the indicated patient during each phase of the study. Open symbols represent measurements below the limit of detection (1 copy/ml). Levels of residual viremia during intensification were not statistically different from pre- and post-intensification levels.

The third project is focused on the dynamics of the appearance and disappearance of drug resistance mutations in HIV-infected individuals, using assays developed in the Virology Core that are capable of detecting mutations with a frequency of >0.03%. In several collaborative studies, we are assessing the role of low-frequency mutations on subsequent treatment failure and analyzing the appearance and disappearance of NNRTI resistance mutations in women exposed to single-dose nevirapine to prevent mother-to-infant transmission.

The goal of the fourth project is to improve our understanding of HIV-1 drug resistance *in vivo*. In a clinical protocol of patients with drug-resistant HIV-1, we are investigating the effects of short-duration, single-drug discontinuations. Using standard viral RNA level determinations and single-genome sequencing, we are investigating the contribution of individual antiretrovirals to partial suppression of virus, and identifying mutations associated with resistance to that drug. We are currently investigating the role of new RT connection domain mutations identified by Dr. Vinay Pathak of the HIV Drug Resistance Program.

Selected Publications:

1. Maldarelli F, Palmer S, King MS, Wiegand A, Polis MA, Mican J, Kovacs JA, Davey RT, Rock-Kress D, Dewar R, Liu S, Metcalf JA, Rehm C, Brun SC, Hanna GJ, Kempf DJ, Coffin JM, Mellors JW. ART suppresses plasma HIV-1 RNA to a stable set point predicted by pretherapy viremia. *PLoS Pathog.* 3: e46, 2007.
2. Palmer S, Maldarelli F, Wiegand A, Bernstein B, Hanna GJ, Brun SC, Kempf DJ, Mellors JW, Coffin JM, King MS. Low-level viremia persists for at least 7 years in patients on suppressive antiretroviral therapy. *Proc. Natl. Acad. Sci. USA* 105: 3879-3884, 2008.
3. Wilkin TJ, McKinnon JE, Dirienzo AG, Mollan K, Fletcher CV, Margolis DM, Bastow B, Thal G, Woodward W, Godfrey C, Wiegand A, Maldarelli F, Palmer S, Coffin JM, Mellors JW, Swindells S. Regimen simplification to atazanavir-ritonavir alone as maintenance antiretroviral therapy: Final 48-week clinical and virologic outcomes. *J. Infect. Dis.* 199: 866-871, 2009.
4. Dinoso JB, Kim SY, Wiegand AM, Palmer SE, Gange SJ, Cranmer L, O'Shea A, Callender M, Spivak A, Brennan T, Kearney M, Proschan MA, Mican JM, Rehm CA, Coffin JM, Mellors JW, Siliciano RF, Maldarelli F. Treatment intensification does not reduce residual HIV-1 viremia in patients on highly active antiretroviral therapy. *Proc. Natl. Acad. Sci. USA*, 9: 106-123, 2009.

Mary Kearney, Ph.D.

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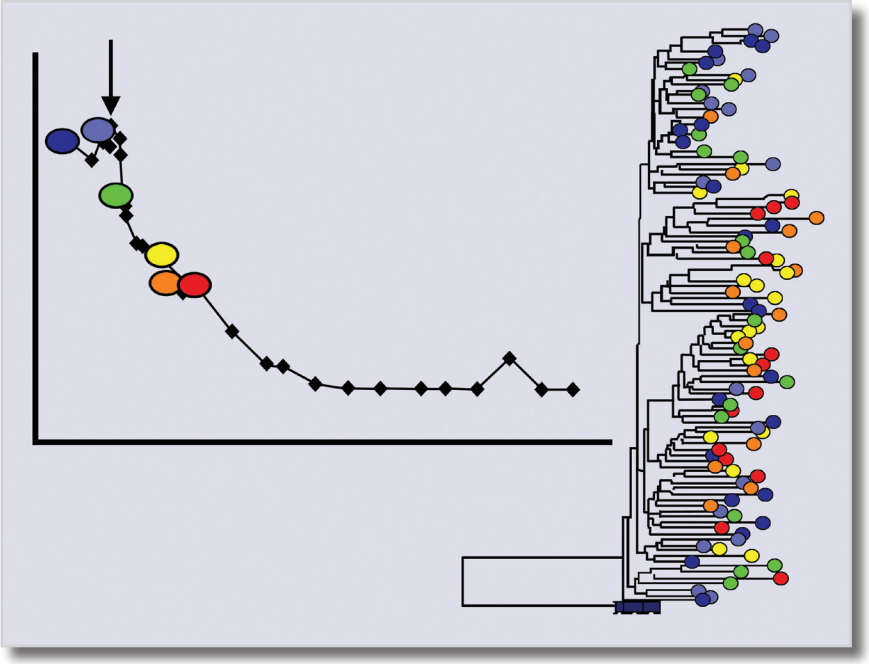
Research Interests:

- **Development of novel clinical HIV-1 assays**
- **Generation of HIV-1 genetic diversity**
- **Detection of minority HIV-1 drug resistance mutations**
- **Persistent viremia in patients on and off antiretroviral therapy**

Biography:

Dr. Mary Kearney received her Ph.D. in Biology at Catholic University in 2007 under the direction of Drs. John Coffin, Sarah Palmer, and Venigalla Rao. In 2008, she was awarded the Benedict T. DeCicco Award for Excellence in Graduate Research by the Biology Faculty of the Catholic University of America. Dr. Kearney joined the HIV Drug Resistance Program in 2001 as a Biologist in the Virology Core and was promoted to Manager of the Virology Core in 2008. In her current position, Dr. Kearney oversees a team that is developing novel analyses for the virological studies undertaken within the HIV Drug Resistance Program, as well as performing standard analyses of HIV in both clinical and basic studies. Her research is focused on developing methods for obtaining very large numbers of sequences from virus circulating in plasma, for measuring virus load with much greater sensitivity than can be reliably attained with currently available tests, and for detecting minor drug resistance mutations *in vivo*.

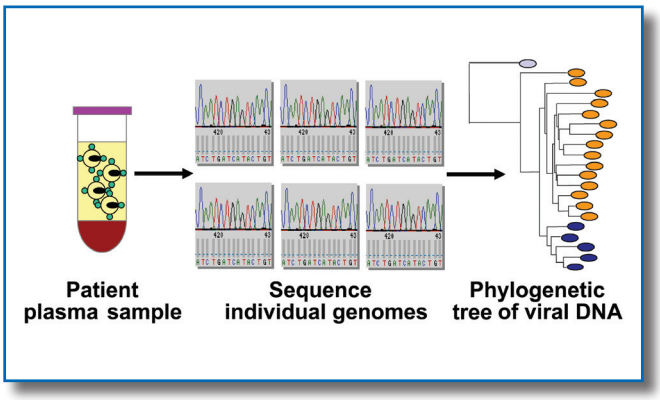


Studying the genetic relatedness of multiple single-virion amplicons by using a limiting-dilution RT-PCR technique provides useful insight into the evolution of genetic diversity of HIV-1 populations. The figure illustrates that chronically infected patients initiating treatment have highly diverse populations prior to therapy. Even though rapid viral load declines occur after initiation of treatment (shown by the arrow), phylogenetic analysis has revealed that HIV-1 population structure post-treatment is indistinguishable from pretherapy samples, even following greater than 100-fold decreases in plasma HIV-1 RNA levels. These results suggest a large replicating HIV-1 population in infected persons.

Virology Core

The Virology Core is primarily responsible for supporting the clinical research efforts of the HIV Drug Resistance Program by developing and applying new technologies to quantify low-level viremia, to detect low-frequency drug-resistant mutants, and to characterize the genetic diversity of HIV-1 in infected individuals. The Core has developed the following assays for application to projects within the Host-Virus Interaction Branch and other sections of the HIV Drug Resistance Program:

- **Single-Copy Assay (SCA) for Detection of HIV-1 RNA in Plasma.** Potent antiretroviral therapy is effective in suppressing, but not eradicating, HIV-1 infection. Persistent viremia can be detected in patients on antiretroviral therapy despite suppression of plasma HIV-1 RNA to <50 copies/ml. The Core developed the SCA for detection of HIV-1 RNA, which has a dynamic range of 10^6 to 0.3 copies/ml. This technology is now being applied to characterize the prevalence, level, and change over time of persistent viremia.
- **Allele-Specific PCR (ASP) for Detection of Low-Frequency Mutations.** Low-frequency drug-resistant mutants are hypothesized to contribute to antiretroviral treatment failure. The Core developed the ASP assay for quantifying drug-resistant HIV-1 variants at reverse transcriptase amino acid codon positions 103, 181, 184, and 190 at frequencies as low as 0.1%. The Core is applying the ASP assay to plasma samples from treatment-naïve and experienced patients to assess the frequency of mutant alleles and their relation to virologic response to antiretroviral treatment.



Single-genome sequencing assay developed by the Virology Core for the genetic analysis of HIV-1 populations.

- **Single-Genome Sequencing (SGS) for Genetic Analysis of HIV-1 Populations.** Characterization of the genetic diversity of HIV-1, including the prevalence of rare drug-resistant variants, in patients with acute infection or chronic untreated infection and in patients initiating antiretroviral therapy can provide important insights into the effective population size of HIV-1, and the likelihood of emergence of drug-resistant variants. Standard population-based DNA sequencing methods cannot reliably detect HIV-1 variants that comprise <20% of the virus population. Furthermore, these methods cannot distinguish whether alleles are present on the same genome or different genomes. The Core developed the SGS assay for detecting sequences derived from individual viral genomes in plasma. Using the SGS assay, the Core has studied HIV-1 diversity and divergence in recently infected patients followed for up to 5 years after infection, in patients who were chronically infected with HIV-1 for 5 years and longer, and in HIV-1-infected patients initiating therapy.

Selected Publications:

1. Maldarelli F, Palmer S, King MS, Wiegand A, Polis MA, Mican J, Kovacs JA, Davey RT, Rock-Kress D, Dewar R, Liu S, Metcalf JA, Rehm C, Brun SC, Hanna GJ, Kempf DJ, Coffin JM, Mellors JW. ART suppresses plasma HIV-1 RNA to a stable set point predicted by pretherapy viremia. *PLoS Pathog.* 3: e46, 2007.
2. Kearney M, Palmer S, Maldarelli F, Shao W, Polis MA, Mican J, Rock-Kress D, Margolick JB, Coffin JM, Mellors JW. Frequent polymorphism at drug resistance sites in HIV-1 protease and reverse transcriptase. *AIDS* 22: 497-501, 2008.
3. Kearney M, Maldarelli F, Shao W, Margolick JB, Daar ES, Mellors JW, Rao V, Coffin JM, Palmer S. Human immunodeficiency virus type 1 population genetics and adaptation in newly infected individuals. *J. Virol.* 83: 2715-2727, 2009.
4. Dinoso JB, Kim SY, Wiegand AM, Palmer SE, Gange SJ, Cranmer L, O'Shea A, Callender M, Spivak A, Brennan T, Kearney M, Proschan MA, Mican JM, Rehm CA, Coffin JM, Mellors JW, Siliciano RF, Maldarelli F. Treatment intensification does not reduce residual HIV-1 viremia in patients on highly active antiretroviral therapy. *Proc. Natl. Acad. Sci. USA*, 9: 106-123, 2009.

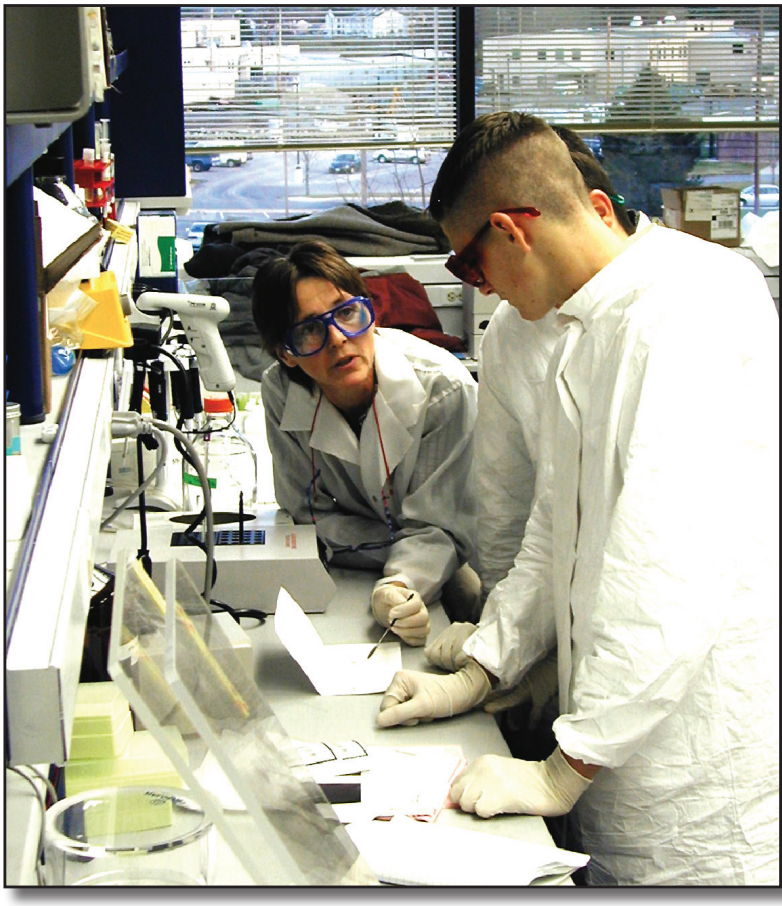
Training and Employment Opportunities in the HIV Drug Resistance Program

The HIV Drug Resistance Program is committed to providing excellent research training opportunities for postdoctoral and postbaccalaureate graduates, college undergraduates, and high-school students. The Program's successful mentoring of young scientists has resulted in former trainees obtaining major positions in academia, industry, and government research programs.

Postdoctoral research training is supported primarily through two fellowship programs: the Cancer Research Training Award (CRTA) provides funding for postdoctoral fellows who are U.S. citizens or permanent residents, whereas the NIH Visiting Fellow Program, administered by the Division of International Services, provides postdoctoral awards to foreign nationals on J1 visas. To be eligible to apply for either program, candidates must hold a doctoral degree or equivalent in the health sciences and have no more than 5 years of relevant research experience since receipt of their most recent doctoral degree. These awards are generally granted for 2 years, with 1-year extensions up to 5 years based on performance and availability of funds. More detailed information about both types of postdoctoral training programs, including a description of current postdoctoral openings by Principal Investigators in the HIV Drug Resistance Program, is available at http://www.training.nih.gov/postdoctoral/.

For postbaccalaureates, college undergraduates, and high-school students interested in pursuing biomedical careers, a variety of programs provide excellent research training opportunities, ranging from summer laboratory internships to 1- to 2-year fellowships. Details about all of these programs may be found at the NIH Research and Training Opportunities website: http://www.training.nih.gov/student/.

In addition to postdoctoral and student research training opportunities, the HIV Drug Resistance Program periodically recruits for technical positions. For further information about all of the recruitment activities in the HIV Drug Resistance Program, please visit http://home.ncifcrf.gov/hivdrp/recruitment.html.



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