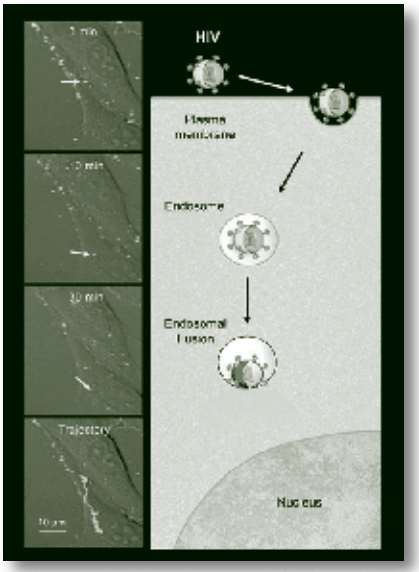
A Compelling New Mechanism for HIV Cellular Entry

Viruses are generally thought to enter cells by one of two mechanisms: they either fuse directly with the cell's plasma membrane, or they are taken up by the cell in a process called endocytosis, and fuse with compartments called endosomes. While HIV has so far generally been considered to use the first mechanism, evidence for this has been mostly indirect, and it is still not clear exactly how HIV infects cells.

Recent research from IHV sheds new light on the topic. The study, published in the May 1 issue of the journal Cell, shows that at least in some cells HIV needs to undergo endocytosis to deliver its contents into the cell. "This is important, definitive work, showing that at least in some cell lines HIV is primarily endocytosed," says Dr. Robert Gallo, Director of the Institute of Human Virology (IHV) at the University of Maryland School of Medicine.

According to Dr. Gregory Melikyan, the IHV researcher who served as senior author on the study, "we were basically interested in the mechanism of fusion between the HIV envelope and cellular membranes. What I realized was that the tools were not in place to answer this question."

Melikyan and his colleagues (Kosuke Miyauchi, Yuri Kim, Olga Latinovic and Vladimir Morozov) took two major approaches in their study. In one, they optimized a technique to trap a bacterial enzyme, beta-lactamase, inside the viral particle. They could detect when this enzyme was delivered into the cytosol. They then used 2 inhibitors – a peptide that inhibited the fusion of the virus to the plasma membrane, and a low-temperature block that inhibited all fusion – to identify where the virus fused.



A drawing (right) and images (left) depict the HIV-1 entry route via endocytosis and fusion with an endosomal membrane. A single virus co-labeled with membrane and content markers moves toward the cell nucleus and releases its content into the cytosol. The bottom image shows the virus trajectory.

According to Melikyan, "if all fusion events occur at the cell surface, no matter whether you apply the peptide or the low temperature you will get identical results." What he found, however, was that viruses get quickly taken up in endosomes, where they are protected from the peptide. They then remain there, still susceptible to the low-temperature for a considerable time before they fuse.

This was an unexpected result, and to confirm it Melikyan and his colleagues developed their second approach, a way to directly image fusion of a single viral particle. "We labeled both the envelope membrane and the interior of the virus." If the virus delivers its contents into the cell, the green fluorescent protein used to mark the interior of the virus escapes into the cytosol and disappears. Similarly, when the virus fuses to the plasma membrane, the dye present on the viral envelope disappears. However, this dye remains visible when the virus fuses with endosomes. So there's a clear-cut difference when the virus fuses at different locations, says Melikyan.

After looking at thousands of viral particles, "we saw to our surprise that real fusion or content delivery, which is the surrogate of infectivity, occurs only in endosomes," says Melikyan. On the cell surface, HIV seemed to undergo only partial fusion, involving exchange of lipids and activation of fusion proteins, but never completing the content delivery process.

The fact that the virus was unable to infect through the plasma membrane led Melikyan and his colleagues to consider the possibility that HIV might require cellular co-factors present in the endosomes to complete fusion. In fact, when they tested dynamin, a cellular protein normally involved in endocytosis, they found that it seemed to be necessary for the virus to fuse with endosomes and release its contents into the cytosol.

This sets the stage for many future experiments to try to understand the cell biology that is going on, says Melikyan. "We have to try to come up with creative ways of knocking dynamin down and going after dynamin partners, because it may not be directly involved." The approaches used in the study are also applicable to other viruses. According to Melikyan, "there are indications that for many viruses, depending on cell types, there is a block for fusion on entry at the (cell) surface." Another important step would be to repeat the experiments in other cell lines. Melikyan also plans to extend their approach to primary T-cells, the major targets of HIV in human infections.

The study's finding that HIV appears to get quickly endocytosed also has implications for anti-HIV drugs. Gallo points out, "if this proves to be true in primary cells, treatment and vaccine research need to be reevaluated to keep this in mind. This could lead to new approaches." "Once the virus is inside, most inhibitors are not getting to the virus anymore," says Melikyan. His study suggests that some drugs could be more potent if endocytosis was delayed. The fact that the virus appears to spend more time inside the cell also raises the possibility of developing membrane-permeable inhibitors to target the virus in endosomes. Melikyan adds, "of course if dynamin or other cellular partners are involved, then hypothetically speaking one could go after cellular targets like that to minimize infection."

Grants

Alash'le Abimiku

Alash'le Abimiku, PH.D., Research Assistant Professor, Institute of Human Virology, received a one-year $268,595 National Institutes of Health agreement in consortium with Vanderbilt University and from the Center for Disease Control for her work entitled, "Implementation of Programs for Prevention, Care and Treatment of HIV/AIDS in the Federal Republic of Nigeria Under PEPFAR."

Manhattan Charurat

Manhattan Charurat, PH.D., Assistant Professor, Institute of Human Virology, received a four-year $2,867,893 National Institutes of Health grant from the National Institute of Allergy and Infectious Diseases for his work entitled, "Acute HIV Infection and Pregnancy." Also on the grant are key personnel from IHV including William Blattner – other significant contributor, Alash'le Abimiku - co-investigator, and Jean Carr –investigator.

Wuyuan Lu

Wuyuan Lu, PH.D., Associate Professor, Institute of Human Virology, received a one-year $45,000 grant from the CRF Program Pilot Grant competition for FY2009 for his work entitled, "Discovery of D-Peptide-Based p53 Activators for Anticancer Therapy," University of Maryland Marlene and Stewart Greenebaum Cancer Center.

Gregory Melikyan

Gregory Melikyan, PH.D., Associate Professor, Institute of Human Virology, received a two-year $375,000 National Institutes of Health grant from the National Institute of Allergy and Infectious Diseases for his work entitled, "Functional Characterization of the Hepatitis C virus E1-E2 Glycoproteins." This funding is supported by the American Recovery and Reinvestment Act of 2009 (stimulus funding).

Dave Pauza

Dave Pauza, PH.D., Assistant Director, Institute of Human Virology, received a four-year $1,238,776 National Institutes of Health grant from the National Cancer Institute for his work entitled, "Mechanisms for depleting tumor immunity in AIDS." Also on the grant are key persons: Cristiana Cairo, Investigator (IHV) and Andrei Chapoval, Investigator, Assistant Professor, Department of Otorhinolaryngology-Head and Neck Surgery, SOM.