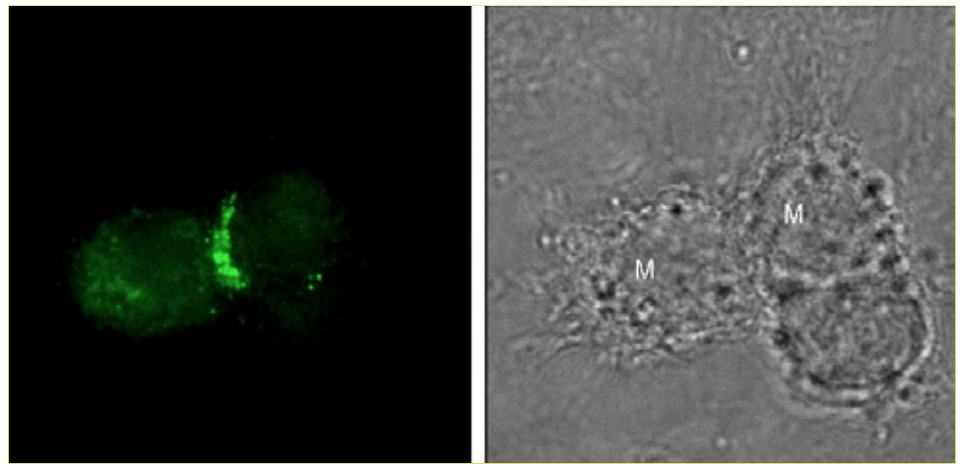


Revealing the Gag Itinerary: How HIV Is Transmitted One Cell at a Time

Although person-to-person transmission of HIV has been well understood for years, researchers are still trying to figure out exactly how the virus moves from one cell to another. Researchers have delineated the steps and key players involved in the generation of HIV particles inside infected (host) cells. However, the precise location of virus assembly inside these cells and the route the virus takes to reach the cell surface before it is released to infect other cells are still matters of heated debate.

A protein called Gag is indispensable for the assembly of HIV particles and plays a role in forming connections between infected and uninfected cells. When a person is infected by HIV, the first cells the virus invades are immune cells called macrophages. These cells, which can be developed from white blood cells in the laboratory, are frequently used to study the nature of HIV-1 assembly. Previously, researchers observed “newborn” viruses in bubble-shaped sacs called endosomes, which are normally used to transport large molecules between specific sites inside a cell. However, the itinerary of HIV trafficking inside macrophages is poorly understood.

In an attempt to shed light on this matter, Eric O. Freed, Ph.D., Head of the Virus-Cell Interaction Section of



These two pictures show Gag accumulating at the macrophage-viral synapse. The macrophage on the right (indicated by the letter “M” in the right panel) is infected with HIV-1 bearing a tetracycline tag, which allows the Gag protein to be visualized (in green). The image shows the high-level accumulation of Gag at the junction between the infected cell (right) and the uninfected cell (left).

NCI’s HIV Drug Resistance Program, and Karine Gousset, a Post-Doctoral Fellow in the program, conducted a study with collaborators from the University of Michigan and NCI’s AIDS Vaccine and Advanced Technology programs at SAIC-Frederick, Inc. The results of their work were recently published in *PLoS Pathogens*.

To visualize trafficking of Gag proteins in real time, Freed and colleagues inserted a small molecular tag—tetracycline (TC)—into the HIV-1 Gag protein. A cellular stain that becomes fluorescent upon binding to the TC tag was applied so that Gag’s position could be tracked by fluorescence microscopy. It was determined that Gag

accumulates at the cell membrane and in compartments in the interior of infected macrophages. Remarkably, when uninfected macrophages or T cells were added, the researchers were able to observe migration of fluorescent Gag to the points of cell-cell contact, so called “virological synapses.” Electron microscopy analysis demonstrated budding of viral particles at the synapse.

Recent work suggested that one of the four components of Gag—the MA domain—regulates targeting of nascent viruses to the plasma membrane of the host cell. In their present study, the authors confirmed that the MA domain of Gag is required for its

recruitment to the synapse. Surprisingly, Gag proteins do not move about in an infected cell unless the cell is engaged in active contact with an uninfected cell. Once it arrives at the synapse, Gag can be seen moving along the surface of the macrophage.

This study demonstrates that HIV-1 particles are retained in internal reservoirs from which they can be

rapidly released at opportune times, such as when contact is established with uninfected cells. The TC system provides an efficient way of observing Gag trafficking in living cells without disrupting the normal virus assembly and release and is likely to become an important tool in identifying molecular clues for HIV-1 trafficking within and between cells. The results of this study may set the groundwork for the

development of new HIV treatments based on interruption of intracellular viral trafficking.

Reference

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(3): e1000015, 2008.