Hoping for a fluorescent basket case: How HIV is assembled and released from infected cells

November 12 2009

Although recent advances have raised hopes that a protective vaccine can be developed, acquired immunodeficiency syndrome (AIDS) remains a major public health problem. Much has been learned about HIV-1, the virus that causes the disease. However, basic aspects of person-to-person transmission and of the progressive intercellular infection that depletes the immune system of its vital T cells remain imperfectly understood.

In a paper published today in the online journal PloS Pathogens, Professor Don Lamb's group at the Ludwig-Maximilians-Universitaet (LMU) in Munichs's Department of Chemistry and Biochemistry, together with colleagues in Heidelberg, describe in detail how new virus particles assemble at the membrane of infected cells, and are released to attack healthy cells nearby. The new findings could help provide clues as how to interrupt the process of intercellular viral spread.

As many of us have learned from personal experience, computer viruses, which contain short pieces of malicious code and arrive in anonymous packages, can gum up data-processing routines. This definition also fits their biological counterparts, which generally comprise compact genomes packed in protein shells, and enter cells via specific portals. For example, the retrovirus HIV-1 has only nine genes in its RNA genome and infects cells by binding to specific receptors. Inside the cell, the genetic material is copied and 15 viral proteins are synthesized. They interact to pack the genomic RNA into new viral particles. These are

then extruded from the cell, wrapped in an envelope of membrane bearing viral proteins that direct the parcel to the next susceptible cell.

The basket that encases the viral RNA is constructed from the Gag protein. Gag is highly versatile: It can bind to the inner face of the cell membrane, to the viral RNA, to itself (to form the shell around the RNA) and to cellular proteins that extrude the newly assembled particle into the extracellular medium. Indeed, Gag can form virus-like particles in the absence of other viral proteins. For their experiments, Professor Lamb's team used cultured cells containing eight of the HIV-1 genes, one of which coded for a fluorescent form of Gag.

"We adopted our custom-built microscope specifically for the experiment, visualizing Gag in the cellular plasma membrane by Total Internal Reflection Fluorescence Microscopy while alternately switching to Wide-Field Fluorescence Microscopy to get a deeper view into the cell", explains Lamb. This allowed the team to track single Gag particles and follow the assembly process, in real time.

Once virus assembly is switched on within an infected cell, the membrane surface of the cell becomes covered with viruses in one to two hours. Each virus is assembled individually at the plasma membrane on the time scale of minutes, rejecting the idea of a reusable assembly platform that is believed to exist for other viruses. By tracking individual viruses, the scientist could follow the processes of assembly from initiation of assembly through to release, learning that it takes about 25 minutes to produce an HIV virus. Hence, a lag of 15-20 minutes precedes release of the enveloped virus, presumably because it takes time for the hijacked cellular budding machinery to close of the virus and release it into to the extracellular medium.

"Using a 'photoconvertible' version of the famous green fluorescent protein - whose discovery and utilization in biological systems were

honored with the Nobel prize in chemistry in 2008 - attached to the Gag protein, we were able to convert the color of membrane bound Gag proteins from green to red", says Lamb. "Thereby, we could determine that viruses were assembly from protein delivered directly from the cytosol or had only arrived recently to the plasma membrane." The new findings add an important dynamic dimension to the process of intercellular viral spread. If they help find ways to interrupt it, HIV-1 could finally be stamped as "undeliverable". (PH)

More information: "Dynamics of HIV-1 assembly and release", Sergey Ivanchenko, William J. Godinez, M. Lampe, H.G. Kräusslich, R. Eils, K. Rohr, C. Bräuchle, B. Müller, D.C. Lamb, *PLoS Pathogens*, 6 November 2009

Source: Ludwig-Maximilians-Universität München