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Novel regulatory step during HIV replication

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A previously unknown regulatory step during human immunodeficiency (HIV) replication provides a potentially valuable new target for HIV/AIDS therapy, report researchers at the Salk Institute for Biological Studies and the University of Wisconsin, Madison.

Their study, published in this week's early online edition of the Public Library of Science, PLoS Pathogens, describes a new biological function for sulfonation—a type of chemical modification—which ensures that viral genes can be expressed efficiently after HIV successfully integrated into the host genome.

"The early steps of HIV infection are highly dependent on cellular processes and represent a time when the virus is particularly vulnerable to antivirals and host defense mechanisms," said John Young, Ph. D., a professor in the Infectious Disease Laboratory at the Salk Institute, who co-led the study with Paul Ahlquist, a Howard Hughes Medical Institute investigator and professor at the University of Wisconsin, Madison. "Drugs that block the sulfonation pathway might render host cells resistant to HIV infection," adds Ahlquist.

HIV begins its assault by injecting its core that contains a single-stranded RNA into a host cell. Once inside, the viral RNA is converted into double-stranded DNA—a process known as reverse transcription—and the original viral RNA is degraded. Another enzyme, integrase, mediates the final step of the genome conversion, where the viral double-stranded DNA slips into the host's DNA, allowing it to take advantage of the host cell's genetic machinery to replicate and propagate itself.

During each step along the way, the virus, which only brings along a handful of proteins, relies heavily on its host cell to pitch in but the roles played by cellular factors are only partially understood. To identify cellular processes that participate in these critical steps, postdoctoral researcher and first author James W. Bruce, Ph.D., infected cells with retroviruses, which inserted themselves into the genome, disrupting the function of individual genes.

He then screened the mutagenized cells for their ability to resist infection with murine leukemia virus (MLV), a virus often used as a model system for HIV. The molecular design of the viral squatters allowed him to identify the genes whose inactivation made it difficult for MLV to multiply within its host cell. One of them was the gene coding for PAPSS1, short for 3'-phosphoadenosine 5'-phosphosulfate synthase 1.

"PAPSS1 is not only part of an important cellular pathway," says Bruce, who divides his time between Young's and Ahlquist's lab, "but it can be shut down with readily available chemical inhibitors, which made it a very attractive target for potential therapeutics."

PAPSS1 and the closely related PAPSS2 synthesize PAPS, whose job it is to provide the sulfonate group used in all sulfonation reactions. Sulfotransferases then move the sulfonate group from PAPS onto proteins, carbohydrates, and lipids. Sulfonation is also involved in detoxification, hormone regulation, and drug metabolism.

Further experiments with chemical inhibitors of PAPS synthases and cellular sulfotransferases confirmed the importance of the cellular sulfonation pathway for retroviral replication. "We knew that certain HIV co-receptors on the cell surface are sulfonated and that this was important for viral entry but we had no idea that sulfonation also played an important role during the infectious cycle within the cell," says

Young.

At closer inspection, the virus had no problem getting inside the cell and setting up house. However, if sulfonation was impeded genetically or through chemical inhibitors during or shortly after MLV integration, subsequent gene expression controlled by the viral long terminal repeat (LTR) promoter was compromised. "We found the same level of integrated virus DNA but when we looked at viral gene expression it was 10 to 20 times lower," says Bruce.

LTRs flank the viral genome and function like "sticky ends", which integrase uses to insert the HIV genome into the host DNA. But they also acts as promoters, regulatory regions that interact with cellular and viral factors to trigger gene expression as well as the transcription of the whole genome into RNA copies that are packaged into the next generation of virus particles.

"Activation of the LTR is a major step in triggering HIV replication but we hadn't realized before that it is also subject to regulation at a step that coincides with integration," says Ahlquist. "This discovery might open up new avenues for the development of drugs that specifically target this novel aspect of retroviral biology."

Source: Salk Institute