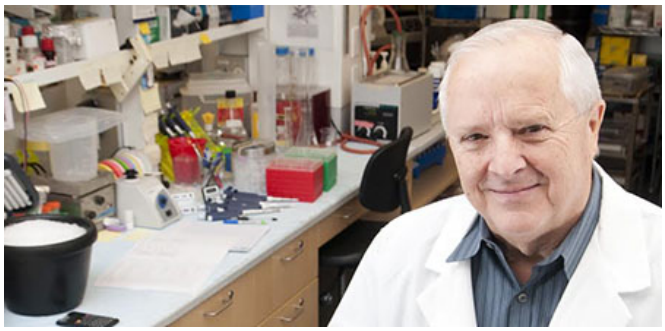
# Trapped: Cell-invading piece of virus captured in lab

6 August 2014



Duane Grandgenett, Ph.D., professor at Saint Louis University's Institute of Molecular Virology, discovered integrase in 1978. Credit: Saint Louis University

In recent research published in the *Journal of Biological Chemistry*, Saint Louis University investigators report catching integrase, the part of retroviruses like HIV that is responsible for insertion of the viral DNA into human cell DNA, in the presence of a drug designed to thwart it.

This achievement sets the stage to use x-ray crystallography to develop complete images of HIV that include integrase, which in turn will help scientists develop new treatments for the illness.

Duane Grandgenett, Ph.D., professor at SLU's Institute of Molecular Virology and senior author of the study, discovered integrase in 1978, little knowing the piece of virus would provide the basis for an entire class of drugs that now treats HIV.

"In 1974, we hadn't heard of HIV yet," Grandgenett said. "We did, however, study retroviruses, the class of viruses that includes HIV. Retroviruses spread by taking over your cell's DNA.

"And the way the virus does this is with integrase. It's responsible for inserting the genetic information of the virus, the DNA, into our chromosomes establishing the viral reservoir. Then, it uses our cells to replicate.

"Integrase is a key component that makes HIV pathogenic."

When a person is infected with HIV, there is an initial burst of virus production. This is when integrase inserts the virus DNA into many human cells, including CD4 T-immune cells, brain cells and other lymph cells. HIV is particularly devastating to the immune system's T-cells, which protect the body from infection.

"Most people do not die from virus replication but from secondary causes," Grandgenett said. "Their immune system collapses and opportunistic infections and cancer are what really kill the person."

Now, scientists have developed drugs that are very successful at managing HIV. Combinational drug therapy is particularly effective. The virus mutates so that it can quickly become resistant to a drug. But when three different drugs aim at three different targets, as in combination drug therapy, the probability of drug resistance is much smaller.

There is one catch, however. Patients must take the drugs every day. If they do not, the virus starts cycling again and within a few weeks the viral levels are back up.

Scientists continue to try to stay a step ahead of the virus, both to combat drug resistance and to develop better treatments.

To develop better drugs, scientists want to use a process called x-ray crystallography to develop a complete picture of how integrase inhibitors – the class of HIV drugs that target integrase— interact with the virus.

"We're aiming to develop newer, better medicines,"

Grandgenett said. "We want to better understand how the integrase inhibitor drugs interact with integrase.

"So far, everybody has failed to produce HIV integrase-DNA images via high resolution x-ray crystallography," Grandgenett said. "No one has ever captured the mother load."

This is Grandgenett's goal.

"Now, we're going after full length integrase protein with DNA," Grandgenett said. "This is what I've wanted to do since 1978, even before HIV was identified."

To do this, Grandgenett and his team, including investigators Krishan Pandey, Ph.D., and Sibes Bera, Ph.D., needed to develop an integrase-DNA complex and then kinetically stabilize the complex in the presence of the drug.

Researchers used a surrogate virus to take a shortcut. Because integrase structures are similar in all retroviruses, Grandgenett tried his approach in Rous sarcoma virus (RSV), whose integrase is more readily manipulated than HIV integrase.

All current clinical integrase inhibitors work in the same way: They block integrase which prevents HIV from replicating. Specifically, they do this by stopping viral DNA strand transfer with STIs – strand transfer inhibitors.

Those inhibitors work by binding three components together: viral DNA; viral integrase; and the drug itself. Before this study, no one had been able to produce a synaptic complex (SC) in solution, the place where these three elements meet.

The researchers developed conditions where the HIV strand transfer inhibitors (STIs) trapped the SC of the surrogate RSV integrase. Grandgenett reports that this experiment is first time anyone has ever captured an integrase-DNA-inhibitor SC in solution.

"We've isolated it and now we want to do x-ray crystallography on it to get a better image of HIV integrase," Grandgenett said. "That's the next step. Hopefully, that crystal structure will better explain how integrase drugs and DNA interact at the nanometer level.

"This will help us to design new drugs. There will be a lot of uses for this information."

Provided by Saint Louis University