How are viruses released from a cell?

Basic research projects often involve narrow, focused aspects of basic processes. One such piece is the process by which certain viruses move from one cell to then spread and infect additional cells.

To replicate, viruses turn host cells into "virus factories." The new viruses then move on to infect other cells and the whole process begins again. The mechanism that allows a virus to be released efficiently from one cell and move to another is the main interest of **Ronald Harty's** laboratory at the School.

Viruses are released from a cell though a process called budding. The virus pushes the lipid membrane of the cell outward so that it enfolds the virus. This budding virus separates from the cell and the new virus particle is ready to infect the next cell.

The mechanism that governs this process is not well understood and scientists have been trying to understand this process of budding as it could help in combating viral diseases. Dr. Harty, assistant professor of microbiology, and his colleagues, two years ago identified a sequence of amino acids that is instrumental in the movement of Ebola virus out of a cell. "We discovered a short sequence of four amino acids contained in the matrix protein of Ebola virus that is instrumental to budding," says Dr. Harty. "If this sequence or motif, as it is called, is changed, the budding process slows down significantly."

This motif not only is present in the matrix protein of the Ebola virus, but also in the matrix proteins of vesicular stomatitis virus (VSV) and HIV. All three viruses are RNA viruses with different characteristics and they represent three different virus families: Ebola is a filovirus, one of the biggest viruses; VSV is rhabdovirus, this family includes rabies; HIV is a retrovirus. The motif is interchangeable between these different virus groups, such that the motif from one initiates budding in another group when implanted in that matrix protein.

"We have found that budding occurs if we move the motif from one place to another on the matrix protein," says Harty. "The protein by itself also causes budding. It is thought that the motif interacts with specific cellular proteins for budding to occur. We are trying to identify these cellular proteins. Because budding doesn't completely cease if the amino acid sequence of the motif is changed, we know other interactions are involved in the process."

Most of the work in the laboratory occurs with VSV, a RNA virus with an RNA genome of about 11,000 bases, considerably smaller than the Ebola virus genome of 19,000 bases. VSV, a reportable disease, afflicts cattle and equines. Symptoms in cattle resemble foot and mouth disease and animals must be quarantined until an accurate diagnosis is made. "Although we work with VSV, we do not work with actual Ebola virus, just with DNA plasmids that make the proteins we are studying," says Harty. "These plasmids are just pieces of DNA that are not infectious. We could not work with Ebola virus or any other lethal virus as that requires the highest grade secure laboratory which we do not have."

The members of Dr. Harty's laboratory working on the Ebola virus project are **Jill Licata**, Ph.D. student and **Dr. Ziying Han**, a post doc. Recently, Dr. Harty and **Dr. Bruce Freedman, V'87**, assistant professor of pathobiology, received a NIH grant to examine Ebola virus proteins that may have channel activities in the cell during budding. These may well be another piece needed in the budding process. It may bring scientists closer to a means that can prevent the spread of virus from cell to cell. Dr. Harty's research on the amino acid motifs is also supported by a second NIH grant.

infection; 30-40% of cats in the NE are carriers for the infection) that they could not overcome because they were immunocompromised.

"Right now we give cyclosporine and prednisolone to our transplant patients to prevent rejection of the new kidney," explains Aronson. "Unfortunately, long term use of these immune system suppressing drugs can make a patient more susceptible to infection and cancer. This has been seen in people and we have also seen it in our transplant patients."

The new drug, CTLA4-Ig, is being used in clinical trials in human transplant patients. It is hoped that it does not have the long-term effects of the drugs currently used. "The drug has been shown to prevent rejection in many research models. Hopefully, its more specific mechanism of action will allow a patient to respond to an infection as well." says Aronson. "We are now investigating the drug in vitro to determine its effect on feline lymphocytes, a group of infection-fighting cells. It appears that the drug suppresses feline lymphocyte proliferation, but allows some cells to remain intact. Some of the cells that remain intact appear to be cells (memory cells) that have previously seen the infectious agent and are present to respond to the infectious agent again. Eventually, we have to determine if the degree of immunosupression seen in vitro using CTLA4-Ig is enough to prevent rejection in an in vivo model."

Aronson hopes the drug will provide an alternative to cyclosporine and prednisolone so that her feline transplant recipients remain able to suppress a latent toxoplasmosis infection and fight of any other infections.

Mason is looking at another factor involved in the immune response to *Toxoplasma gondii*. In Hunter's lab, she is studying the role of c-Rel, a gene that controls the production of the cytokines interferon gamma (IFN-g) and Interleukin 12 (IL-12), both essential for resistance to toxoplasma infections.

"The cytokines are important in resistance to toxoplasmosis," says Mason. "We are studying the immune response to toxoplasma in mice that lack the c-Rel gene. When these mice are infected with *Toxoplasma gondii*, their resistance to the disease is very low. They have normal levels of IL-12 and reduced levels of IFN-g early on during infection. This may allow the parasite to establish itself in the mouse and result in a reduced survival time compared to wild type mice."

To know the role the gene c-Rel plays in the immune response is important as this gene is also involved in producing resistance to Leishmania, another protozoan disease that is devasting to people and animals, and to viral infections. Once this role is completely understood, scientists may be able to develop treatments that reinforce c-Rel and help people and animals overcome these infectious diseases.

Aronson and Mason are just two clinicians at the School who are taking advantage of the proximity of the basic scientists to the clinical facilities; there are many more clinicians, at VHUP and at New Bolton Center, who work closely with bench scientists, incorporating basic science findings into clinical applications.