

## Harnessing an innate protection against Ebola

School of Veterinary Medicine researchers have identified a cellular pathway that keeps Ebola virus from exiting human cells, with implications for developing new antivirals.

### fluorescent microscopic image of two cells being infected with virus

An innate mechanism in human cells may prevent Ebola virus from spreading, according to new Penn Vet–led research. Using powerful confocal microscopy, they tracked the budding of virus–like particles from cells (shown in the filamentous projections in the cell in the upper right) and how autophagy, a “self–eating” cellular process, by which viral proteins are sequestered in vesicles (shown in the cell in the lower left), inhibits virus–like particles from exiting. (Image: Courtesy of Harty lab)

**I**n their evolutionary battle for survival, viruses have developed strategies to spark and perpetuate infection. Once inside a host cell, the Ebola virus, for example, hijacks molecular pathways to replicate itself and eventually make its way back out of the cell into the bloodstream, where it can spread further.

But our own cells, in the case of Ebola and many other viruses, aren’t without defenses. In a study published in the *Proceedings of the National Academy of Sciences* (<https://doi.org/10.1073/pnas.2210690120>), a team led by [University of Pennsylvania](https://www.upenn.edu/) [School of Veterinary Medicine](https://www.vet.upenn.edu/) scientists discovered a way human cells hamper the Ebola virus’ ability to exit.

An interaction between viral and host proteins prompts host cells to ramp up activity of a pathway responsible for digesting and recycling proteins, the team found. This activity, known as autophagy, or “self-eating,” allows fewer viral particles to reach the surface of a host cell, thus reducing the number that can exit into the bloodstream and further propagate infection.

“This interaction seems to be part of an innate defense mechanism,” says [Ronald N. Harty](https://www.vet.upenn.edu/people/faculty-clinician-search/ronaldharty), a professor at Penn Vet and senior author on the study. “Human cells appear to specifically target a key Ebola virus protein and direct it into the autophagy pathway, which is how cells process and recycle waste.”

The investigation emerged from a longtime area of focus for [Harty’s lab](https://www.vet.upenn.edu/research/centers-laboratories/research-laboratory/harty-laboratory): the interaction between the viral protein VP40, found in both Ebola and Marburg viruses, and various human proteins. In the group’s previous work, they’ve found that one area of VP40, known as a PPXY motif, binds corresponding motifs known as WW domains on specific host proteins.

In many instances, this PPXY-WW interaction causes more viral particles to exit the cell in a process called “budding.” But in screening various host proteins thought to play a role in the process, Harty and postdoc Jingjing Liang, the study’s lead author, uncovered some that did the opposite upon binding VP40, causing

budding to decrease. One of these was a protein called Bag3, on which they reported in a *PLOS Pathogens* (<https://doi.org/10.1371/journal.ppat.1006132>) paper in 2017.

Though Ebola is a potentially deadly virus, Harty and colleagues can safely study its workings in a Biosafety Level 2 laboratory, substituting virus-like particles (VLPs) that express VP40 for the virus itself. These VP40 VLPs are not infectious but can bud out from host cells like the real thing.

In the new work, the Penn Vet researchers and colleagues from the [Texas Biomedical Research Institute](https://www.txbiomed.org/) (<https://www.txbiomed.org/>) dug deeper to learn about the mechanism by which Bag3 reduced budding. Bag3 is known as a “co-chaperone” protein, involved in forming a complex with other proteins and chaperoning them on their trip to be digested, ultimately in organelles called autolysosomes, part of the process of autophagy. Using VP40 VLPs, Harty’s group confirmed that VP40 bound to Bag3 and formed the protein complex. When the researchers added a compound that is known to block formation of this complex, they saw VP40 being released; VLP budding activity subsequently increased.

To follow the activity of VP40 in real time, the team used powerful confocal microscopy, labeling each actor of interest with a different fluorescent tag. They observed that Bag3 was involved in sequestering VP40 in vesicles in the cell that would go on to undergo autophagy. Stuck in these vesicles and destined for the cellular “recycling center,” VP40 was unable to move to the cell membrane and bud.

“I think one of the most interesting things that we showed is the selectivity of the cargo,” Liang says. “We show that autophagy doesn’t just happen passively. Bag3 acts through the PPXY-WW interaction to specifically target VP40 to undergo autophagy.”

When the researchers added the drug rapamycin, which enhances autophagy, VP40 sequestration went up and VLP budding went down. Rapamycin works by inhibiting the activity of a pathway governed by a protein complex called mTORC1, a cellular sensor that turns on protein synthesis when a cell needs raw material to grow. The researchers found this pathway appeared to be important in regulating Ebola infection; in experiments with live virus conducted in a Biosafety Level 4 laboratory, they observed that the virus could activate mTORC1 signaling, causing the cellular “factory” to produce materials the virus would need to expand and spread. In contrast, inhibiting mTORC1 with rapamycin directed the virus toward the autophagy pathway, where it would be digested by the cell’s autolysosomes.

“The virus wants the cell growing so it activates mTORC1,” says Harty. “Autophagy does the opposite, keeping the cellular materials in balance.”

Autophagy is important for normal cellular processes, ensuring that the cell doesn’t become cluttered with unnecessary or misfolded proteins and other materials floating around. But this work also suggests autophagy can be harnessed by the body to defend against harmful infection.

“Our conception is that this is part of the arms race between our bodies and the virus,” Liang says. “The virus wants to shape its environment to benefit itself and its own survival, so it evolved to manipulate mTORC1. But the cell can also use this pathway to defend against viral infection.”

With these insights into the human body’s innate defenses against Ebola, the researchers hope to see if autophagy may be a factor in other hemorrhagic viral infections, such as those that cause Marburg and Lassa fever. And while the current experiments were primarily conducted using human liver cell lines, the team

would also like to test whether autophagy and the mTORC1 pathway are involved in viral defense in other cell types, such as the immune system's macrophages, the primary cells involved in propagating infection.

Ultimately, Harty, Liang, and colleagues hope to find as many viral vulnerabilities as possible, helping inform drugs that could be one component of a therapeutic cocktail, each targeting different stages of infection, from viral entry to exit.

“This all ties together in our overall goal of understanding viral-host interactions and, by understanding them, working to intervene to slow or stop infection,” Harty says.

*Ronald N. Harty is professor of pathobiology and microbiology at the University of Pennsylvania School of Veterinary Medicine.*

*Jingjing Liang is a postdoctoral fellow in Penn's School of Veterinary Medicine.*

*Harty and Liang's coauthors were the Texas Biomedical Research Institute's Marija A. Djurkovic and Olena Shtanko. Liang was lead author on the study and Harty was corresponding author.*

*The work was supported in part by the National Institutes of Health (grants AI138052, AI139392, AI153815, and EY031465 to Harty and AI154336 and AI151717 to Shtanko.)*

---

#### CREDITS

**Katherine Unger Baillie**  
Writer

---

#### DATE

**February 2, 2023**

---

#### SUBTOPICS

**Infectious Diseases, Immunology, Biology, Veterinary Medicine, Research**

---

#### SCHOOLS

**School of Veterinary Medicine**