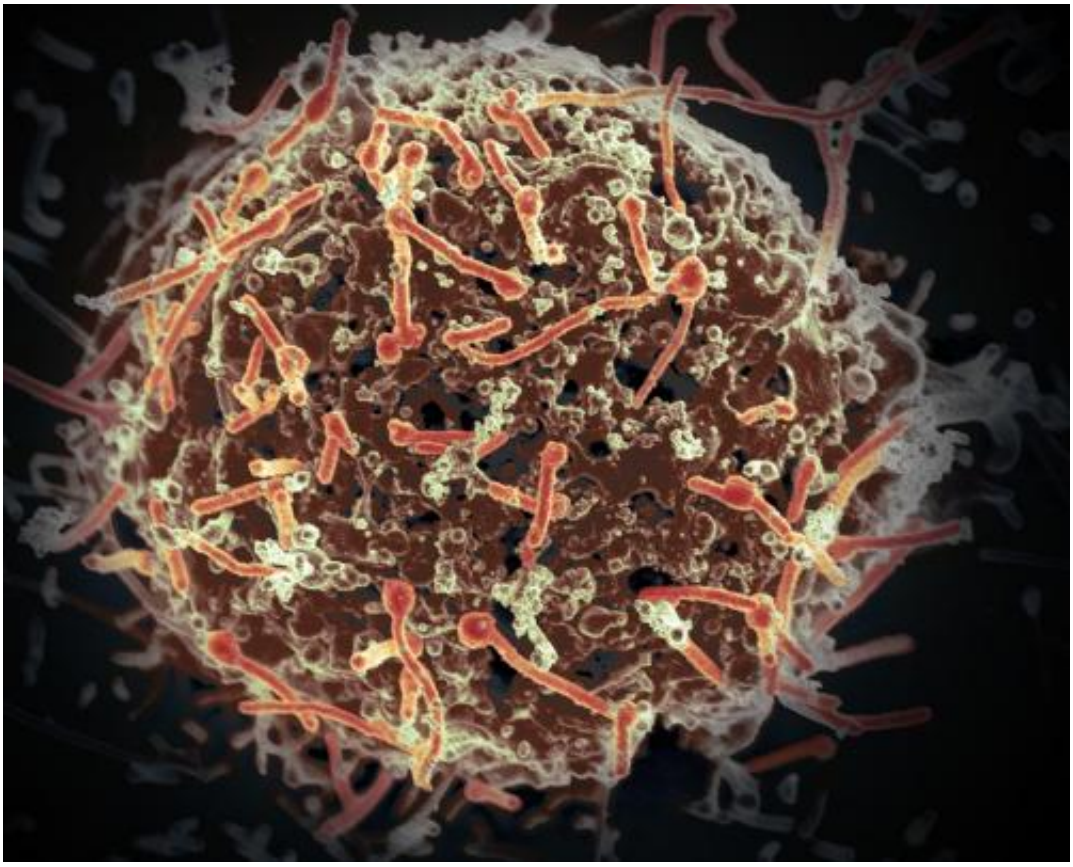
Mutating Ebola's key protein may stop replication

March 12 2018, by Kayla Zacharias



The Ebola virus, isolated in November 2014 from patient blood samples obtained in Mali. The virus was isolated on Vero cells in a BSL-4 suite at Rocky Mountain Laboratories. Credit: NIAID

Researchers may be able to stop the replication of Ebola virus by mutating its most important protein, according to a paper published in

the *Journal of Biological Chemistry*.

Researchers were able to mutate Viral Protein 40 (VP40) in a way that changed the residues of the protein, blocking the budding and replication of Ebola virus in a model system.

VP40 is a peripheral membrane protein that regulates viral budding from the plasma membrane. It interacts with a human plasma-membrane lipid, phosphatidylserine, to facilitate replication of the virus. All animal viruses have to cross membranes for cell entry and exit.

The research team, led by Robert Stahelin of Purdue University, found the specific parts of VP40 that bind with the lipid: a cationic patch on the end of an amino acid chain. This site controls the ability of the protein to form a lipid envelope, the layer that protects the virus from the outside environment.

Water-attracting residues at this site are critical for membrane penetration and budding. Substituting those residues with alanine, which is hydrophobic, reduced lipid binding by 40-fold and stopped localization to the plasma membrane.

VP40 is a transformer protein, capable of rearranging itself into different structures: monomer, dimer and octamer. These various structures interact with the lipid differently, according to the paper. The dimer is best equipped to facilitate replication, performing twice as well as the monomer, and nearly 10 times better than the octamer.

"It's exciting to learn that these different oligomeric structures bind differently with the human lipid cells," Stahelin said. "That might explain why there are different roles for this protein in the viral replication cycle."

There are currently no FDA-approved vaccines or therapeutics available for Ebola virus. Outbreaks are rare but deadly, with fatality rates as high as 90 percent. Knowing how and where the protein interacts with the lipid could allow researchers to better target it with therapeutics.

"This helps us understand how the virus uses human cell membranes to replicate and form new virus particles. The virus needs this one lipid to form the new particle and infect other cells," Stahelin said. "We've been targeting human cells with therapeutics that modulate the way the cell makes lipids, and we like to target the human cell because it isn't likely to mutate and become resistant to the drug.

Cellular and in vitro models were used in this study. In vitro models were used to quantify how well VP40 binds to synthetic membranes. The researchers mutated the DNA code to change the amino acid sequence of VP40, purified those proteins to homogeneity and compared their bindings to that of the original VP40.

In cellular experiments, live cell imaging was used to monitor VP40 localization in human cells. The movement of the mutant VP40 and the original VP40 were compared to see how they bind to the human cell plasma membrane, the site of viral replication.

More information: Kathryn Del Vecchio et al. A cationic, C-terminal patch and structural rearrangements in Ebola virus matrix VP40 protein control its interactions with phosphatidylserine, *Journal of Biological Chemistry* (2018). DOI: 10.1074/jbc.M117.816280

Provided by Purdue University