Scientists find potential Achilles' heel on Lassa fever and related viruses

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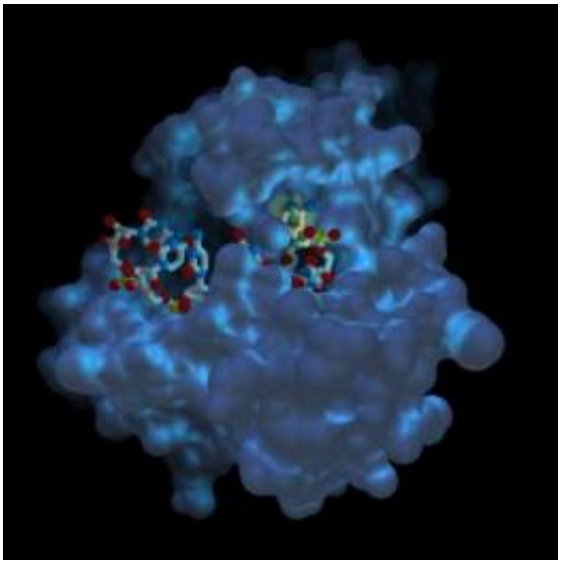

Scientists at the Scripps Research Institute have determined the atomic structure of a protein that the Lassa fever virus uses to make copies of itself within infected cells. This surface representation of the nucleoprotein shows the RNA bound in between the two sub-domains, highlighting, in particular, a deep pocket that could be a prime target for antivirals. Credit: Photo courtesy of the Ollmann Saphire lab, The Scripps Research Institute.

Scientists at The Scripps Research Institute have determined the atomic structure of a protein that the Lassa fever virus uses to make copies of itself within infected cells. The structural data reveal an unexpected molecular crevice where the viral protein grips the viral genes, making this crevice a target for potential antiviral drugs. Lassa fever virus and

other arenaviruses infect hundreds of thousands of people annually and are often deadly. Currently there is no specific therapy or vaccine against them.

"It's the first look we've ever had at an arenavirus nucleoprotein bound to its genome, and so it opens up many new research pathways, and of course gives us a clear aiming point for the development of anti-arenavirus drugs," said Erica Ollmann Saphire, associate professor in Scripps Research's Department of Immunology and Microbial Science. Ollmann Saphire is the senior author of the new report, which appears in an early online edition of the Proceedings of the National Academy of Sciences the week of November 14, 2011.

The arenavirus nucleoprotein serves in part as a scaffold to hold the viral RNA-based genome while it is translated into new viral proteins. Blocking the nucleoprotein's interaction with the viral genome would prevent an arenavirus from replicating itself–and thus should stop the course of an infection.

A separate team of structural biologists reported the atomic structure of the Lassa fever virus nucleoprotein–considered typical of arenavirus nucleoproteins–in 2010. But until now, no one had determined the nucleoprotein structure while bound to viral RNA. "These nucleoproteins can be tricky to work with, because they do bind so easily to RNA or even to themselves," said Kathryn Hastie, a PhD candidate in the Ollmann Saphire lab who performed most of the experiments and is the lead author of the report.

Hastie produced a shortened version of the Lassa fever virus nucleoprotein and, in this way, was able to crystallize it while it was bound to a segment of viral RNA. When a molecule has been crystallized, researchers can beam X-rays at it, record the resulting diffraction patterns, and infer the molecule's 3-D atomic structure.

In this case, the high-resolution structural data revealed the crucial site where the nucleoprotein binds to viral RNA. Previous studies of nucleoproteins from other RNA viruses had predicted the RNA-binding site would be in a central region of the nucleoprotein. However, Hastie, Ollmann Saphire and their colleagues found instead that the Lassa fever virus RNA binding site is within a cleft at one end of the nucleoprotein. "This end of the nucleoprotein is shaped like a clamshell, and in the X-ray diffraction imagery we observed a big strip of positive density sitting between the two halves of the clamshell, which we could identify as RNA," said Hastie.

The structural data and follow up studies indicated that the nucleoprotein normally exists in a "trimer" formation, in which three lengths of the protein are linked in a rough triangle. In this formation, the RNA binding site is normally blocked, but an encounter with another viral protein or some other trigger may "unlock" the trimer formation and expose the RNA binding site.

The Ollmann Saphire lab now is investigating the precise sequence of molecular events that causes the viral nucleoprotein to bind to viral RNA. But it is already clear, for example, that getting a drug into the cleft where the nucleoprotein binds RNA should block RNA-binding and thus stop the virus from replicating. Such a drug might work not only against Lassa fever virus but against other arenaviruses, too. "The part of the Lassa fever virus nucleoprotein that contacts the RNA is exactly the same for every other arenavirus, so it's highly likely that this is how the other arenaviruses bind their RNA," Hastie said.

Ollmann Saphire is now looking for a pharmaceutical company partner to help her lab turn the new finding into a candidate anti-arenavirus drug and to test it clinically.

Lassa fever virus is endemic in parts of West Africa, where its natural

host is a local mouse species. It infects 300,000 to 500,000 people and kills at least several thousand of them annually, according to the Centers for Disease Control. At least five other arenaviruses–Junín, Machupo, Guanarito, Chapare and Sabiá–are found in South America and can cause fatal hemorrhagic fevers. Arenaviruses in North America include the meningitis-associated lymphocytic choriomeningitis virus, which also can silently infect pregnant women and cause birth defects. A recent study found antibodies to arenaviruses in the blood of about 3.5 percent of US patients with neurologic symptoms or fevers of unknown origin.

Arenaviruses usually spread to humans from their rodent host population, but there is evidence that they can spread from human to human, and occasionally they cause large outbreaks of fatal disease. The antiviral drug ribavirin has been used to treat arenavirus disease, but seems to help only modestly, and only when administered in early stages of infection.

"Arenaviruses are a huge class of human pathogens that exist almost worldwide, and they are circulating and evolving continuously in a rodent population that we can never eradicate," says Ollmann Saphire. "We don't know which arenaviruses are only one or two mutations away from causing a major disease outbreak. And up to now we have no real defense against them."

More information: "Crystal structure of the Lassa virus nucleoprotein–RNA complex reveals a gating mechanism for RNA binding," *Proceedings of the National Academy of Sciences* (2011).

Provided by The Scripps Research Institute