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Research captures transient details of HIV genome packaging

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Once HIV-1 has hijacked a host cell to make copies of its own RNA genome and viral proteins, it must assemble these components into new virus particles. The orchestration of this intricate assembly process falls to a viral protein known as Gag. For one thing, Gag must be able to discern viral RNA from the host cell's and squirrel it away inside new viral particles — no easy task considering only two to three percent of the RNA found in the cytoplasm is from HIV-1. Exactly how Gag selectively packages viral RNA has been widely speculated but never directly observed.

Now a team of researchers from Paul Bieniasz's Laboratory of Retrovirology at The Rockefeller University and the Aaron Diamond AIDS Research Center have employed a recently developed technique to capture—in a sort of molecular freeze-frame—just how Gag accomplishes this feat. In research published recently in *Cell*, they reveal that Gag undergoes dramatic and transient changes in binding preferences that allow it to precisely select viral RNA for packaging into new viruses.

"One of the functions of Gag is to choose the viral RNA from all the RNA present in the cell to package into a viral particle, which will then go on to infect a new cell," say Bieniasz. Gag, the major structural protein of HIV-1, floats about as individual molecules in the cytoplasm, but to assemble new viruses, thousands of Gag coalesce at the host cell's plasma membrane, forming an immature viral particle containing two strands of viral RNA.

Previous studies suggested that Gag targeted viral RNA by binding to a sequence known as psi, but many suspected that this interaction alone could not account for Gag's ability to discriminate between viral and host cell RNA.

To observe just how Gag recruits viral RNA, the researchers turned to a technique known as crosslinking-immunoprecipitation (CLIP) sequencing, which uses ultraviolet light to fuse RNA and protein and preserve interactions for further analysis. "CLIP essentially freezes the interaction in space and time, and tells you in a very localized, specific way the RNA sequences your protein was bound to," says first author Sebla B. Kutluay, a postdoctoral fellow in the lab.

Gag does indeed bind to psi on viral RNA, the researchers found, the first time this interaction has been demonstrated in a biologically relevant setting. But as they suspected, there was more to the story. When Gag moves to the plasma membrane, it appears to completely change its behavior and bind to many different sites throughout the HIV-1 genome.

By analyzing the RNA sequences bound by Gag, the researchers discovered that the protein seems to change its taste for nucleotides depending on location. Gag in the cytoplasm prefers RNA sequences rich in guanine, but at the plasma membrane, Gag is temporarily drawn to sequences rich in adenine. Strikingly, the genome of HIV-1 is particularly adenine-rich—an unusual property of the HIV-1 genome that has heretofore puzzled scientists.

Such changes in RNA binding behavior would have been impossible to observe even a few years ago, before the availability of CLIP. "Gag binding to adenine-rich RNAs was never seen before by any approach and could not have been seen by any other approach," says Bieniasz, noting that CLIP was developed by colleagues in Robert B. Darnell's

laboratory and refined in Thomas Tuschl's laboratory at Rockefeller.

The sudden switch in RNA binding appears to be multimerization-dependent—that is, induced by the crowding of Gag at the plasma membrane, which may block certain proteins surfaces and alter binding behavior.

"It's the first example of an RNA binding protein that shows such dramatic changes in specificity depending on where it is in the cell," says Bieniasz. "It really changes the way we understand how HIV packages its genome."

A second major finding, and a surprising one, was that Gag binds also to cellular tRNA. A region of Gag that helps direct the protein to the plasma membrane, known as the matrix domain, binds to tRNA and "it's exquisitely specific," says Bieniasz. Although the researchers cannot say for sure what function is served by tRNA binding, they suggest it might help regulate and pace the process of viral assembly by acting as a temporary shield against unnecessary RNA or membrane binding.

More information: "Global Changes in the RNA Binding Specificity of HIV-1 Gag Regulate Virion Genesis." DOI: 10.1016/j.cell.2014.09.057

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