VIROLOGY

Tracking infection

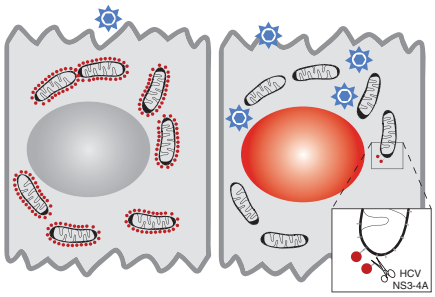
A new cell-based fluorescent reporter system allows direct visualization of individual cells infected with hepatitis C virus in real time.

Hepatitis C virus (HCV) infects liver cells (hepatocytes) and can cause chronic hepatitis. As with other viruses, establishing a system to propagate this human pathogen in cell culture is key to better understand the disease and speed the discovery of specific antiviral therapeutics. Unfortunately, culturing HCV has been especially difficult and is a relatively recent achievement. Even though around 200 million people are affected worldwide, only one isolate from an infected individual has been found capable of propagating in cell culture without acquiring adaptive mutations. Thus, the vast majority of HCV research is currently based on this unique strain as well as on specific hepatoma-derived cell lines required for its growth.

The hardships involved in working with this virus and the need for better tools to monitor infection inspired the group of Charles Rice, at The Rockefeller University, to develop a reporter system in which HCV infection of live cultured cells can be visualized in real time. This system does not require the use of genetically modified viruses and is sensitive enough to allow detection of infectious events in single cells.

The newly developed reporter system is based on the activity of the viral protease, NS3-4A, on a cellular target, IPS-1, that is localized on the mitochondria. "We took advantage of the fact that others in the field had identified a cellular target for the viral protease, and fused its protease recognition site to green or red fluorescent proteins," explains Rice. The authors also included the sequence necessary for mitochondrial localization, so when this construct is introduced into cells, the mitochondria are fluorescently labeled. Upon HCV infection, the viral protease cleaves the chimeric protein and releases the fluorescent signal into the cytoplasm.

The robust signal of this reporter system derives from the efficiency of the NS3-4A cleavage and the high constitutive expression of the substrate, allowing even small amounts of the virus to be visualized. Moreover, the red variants of this reporter assay contain a nuclear localization sequence such that upon virally induced proteolysis the fluorescence moves to the nucleus. "This nuclear translocation increases visualization and has helped a lot with detecting HCV replication in primary cells," explains lead author, Christopher Jones.



In HCV-infected cells (right), the engineered fluorescent protein substrate is cleaved by the viral protease, resulting in translocation of the reporter from the mitochondria into the nucleus. Image courtesy of Charles Rice and Christopher Jones.

In the past, studying HCV infection in primary human hepatocyte cells has been particularly challenging. The authors took advantage of the great sensitivity of their reporter system, as well as a recently reported co-culture platform, to visualize HCV infection of live primary hepatocytes for the first time.

The possible applications of this reporter system are many, as the authors show by adapting the technology to study virus-host interactions and the mechanisms of viral spread. One of the experiments that Rice's group is currently pursuing involves the combination of the reporter with laser-capture microscopy. These experiments will allow infected and uninfected bystander cells to be identified, isolated and compared at the transcriptomic and potentially proteomic levels. With this, the researchers aim to learn more about the changes in cell biology that result from viral infection and find new avenues to explore and interfere with essential virus-host interactions.

This reporter can also be used to screen for new infectious isolates in samples from infected individuals with the hope of expanding the current HCV culture system to other strains, one of the holy grails of HCV research.

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RESEARCH PAPERS
Jones, C.T. *et al.* Real-time imaging of hepatitis C virus infection using a fluorescent cell-based reporter system. *Nat. Biotechnol.* **28**, 167–171 (2010).