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## Catching Poliovirus in the Act

It can take hours, or even days, for a virus to infect a cell. But poliovirus is more efficient than the average virus, new research has shown. Once inside its host cell, poliovirus needs only minutes to release its genome and initiate an infection.

Using fluorescence microscopy to watch as individual polioviruses entered host cells, Howard Hughes Medical Institute researchers at Harvard University have found that the virus must be internalized into a cell in order to release its genome, but once inside, genome release is quick and efficient, and takes place near the cell membrane.

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— **Xiaowei Zhuang**

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The results, published July 9, 2007, in the journal *Public Library of Science Biology*, not only have implications for the understanding of basic virology, but also established a novel method for studying viral entry. The research team was led by Howard Hughes Medical Institute researcher Xiaowei Zhuang at Harvard and Harvard Medical School professor James Hogle.

Despite decades of studying poliovirus, researchers still didn't fully understand how it infected cells. There was a debate about whether this virus releases its RNA right at the cell surface or whether it needs to be internalized first, said Zhuang.

One reason for the debate was that while several hundreds of poliovirus particles may attempt to invade a host cell, few of these actually lead to infection. You would worry about following a large number of virus particles that turn out to be non-infective, Zhuang noted. Then everything you discovered about how they get into the cell is irrelevant.

James Hogle, a specialist on polioviruses, added, Without a proper research design, you never know if you're looking at an infective pathway or not. To overcome this obstacle, the researchers combined studies of viruses' ability to

infect cells with sensitive live cell microscopy techniques that allowed them to track individual polioviruses, each one a thousand times smaller than the cell it infiltrates.

Because they wanted to see how intact poliovirus enters cells, as well as how it releases its genetic material once inside, they tagged the viruses with two different fluorescent dyes. The first bound to RNA, the genetic material carried by polioviruses, and caused it to glow green. The researchers took care to choose an RNA-binding dye that would not interfere with the infectivity of the virus.

They used a different dye to label the protective outer coat of the virus, known as the capsid. They labeled the capsids with a dye that fluoresced in red. In some experiments, the investigators chose a pH-sensitive red dye that fluoresces only at neutral or acidic pH (7 or lower). This way, when the extra-cellular environment was briefly raised to a higher pH, the viruses became invisible. Once a virus entered a host cell, however, the dye would constantly emit red fluorescence, because cells and intracellular organelles typically maintain a pH close to or below 7. Using this approach, the researchers could unambiguously tell whether a virus particle had been internalized into a cell.

Through the microscope, the researchers watched as polioviruses entered host cells, emitting both red and green light, and then released their green-hued RNA. They could see that the virus enters cells through endocytosis, a process in which the cell membrane folds around the virus and creates a sac inside the cell. And by correlating this observation with studies of infectivity, Zhuang said, We settled the debate. We found that the virus does require endocytosis. If the viruses don't get into the cell, they do not release their RNA.

The group also found that viral RNA was released almost immediately after a virus particle entered the cell. Seventy-five percent of polioviruses release their RNA genome in the first 30 minutes, said Boerries Brandenburg, a postdoctoral researcher in Zhuang's lab and first author of the paper.

We found that polio's genome release is efficient, he said. That's not the limiting mechanism of infectivity. There were previous suggestions that it might be.

The group also examined the effects of multiple drugs against the infectivity of the virus. They found that the virus doesn't use any of the known endocytosis pathways to get inside a cell.

We're not the first group to use drugs to test which cellular mechanisms are involved in infection, said Brandenburg. But it's sometimes difficult to find out whether the drugs are affecting infection, or just negatively affecting the cell. Likewise, problems also exist for single-virus tracking experiments in live cells. It is not easy to tell whether the viruses being tracked are the ones that lead to infection. We spent a lot of time on both assays to make sure that our observations are relevant to infection.