

Piecing Together Rotavirus's Unique Approach

An extra protective layer primes this virus to do its harm, mainly in children in the developing world.

FOR A VIRUS TO INFECT THE CELLS OF ITS HOST, IT MUST FIRST find a way in. For some viruses, the strategy is to fuse membranes with the target cell, often after hitching a ride in a vesicle with an entry ticket. In the case of viruses with no membranes of their own, such as rotavirus, the story is more complicated. Using two complementary techniques of structural biology—x-ray crystallography and electron cryomicroscopy—HHMI researchers have uncovered some important details about how rotavirus manages to make its way into a cell.

Rotavirus particles surround their RNA genomes with three protein layers. Understanding the structure and behavior of the proteins that constitute those layers could help scientists design better vaccines for a disease that yearly kills half a million people, most of them children in the developing world. Current vaccines, based on a live, attenuated form of the virus, may be impractical in the world's poorest regions. A protein-based vaccine would be easier to ship, store, and combine with other vaccines, says HHMI investigator Stephen Harrison of Harvard Medical School and Children's Hospital, Boston. With this goal in mind, Harrison studies the virus's outermost coat proteins, which he says are

appropriate candidates for a vaccine because immune system antibodies recognize them.

Despite its multilayered configuration, rotavirus lacks a lipid membrane, or envelope, that can fuse with lipid-containing membranes of host cells. Entry of the so-called nonenveloped viruses is not as well understood as that of their enveloped counterparts. They use a diverse, and still largely unexplored, range of strategies to infiltrate host cells.

Unlike the genomes of simpler viruses, rotavirus RNA always remains enclosed within two of the three protein layers that surround it in the infectious virus particle. Enzymes packaged with the genome make and export new RNA for incorporation

into progeny particles. The outermost layer, acquired before the virus emerges from one infected cell and searches for another, consists of two proteins—VP4 and VP7. The spike-shaped VP4 is thought to perforate the membranes of host cells, but the role of VP7 in penetration has been less clear.

In work published in the June 12, 2009, issue of *Science*, Harrison and his team (which included student Scott Aoki, HHMI postdoctoral fellow Ethan Settembre, and collaborator Philip Dormitzer) crystallized VP7 in the clutch of an antibody and, using x-ray crystallography, determined the molecular structure of the complex. It showed both how calcium ions hold VP7 together as a trimer of three identical molecules and how an antibody can prevent it from coming apart. The investigators concluded that the VP7 trimer must come apart during viral entry and that a loss of calcium ions in the host environment might trigger this process.



The study offered a picture of VP7 in isolation from the virus. To visualize the outer-coat proteins on an intact virus particle, Harrison partnered with Nikolaus Grigorieff, an HHMI investigator at Brandeis University and expert in an emerging technique called electron cryo-microscopy (cryo-EM).

In cryo-EM, scientists preserve a large macromolecular complex by freezing it rapidly in a bath of liquid ethane (see Toolbox, page 44). Once immobilized, particles can be imaged from every angle with a narrow beam of electrons. The thou-

sands of images are then averaged into a high-resolution three-dimensional picture. The symmetrical nature of rotavirus helps, Grigorieff explains, because each image reflects what the structure looks like from multiple angles.

The result was a picture that showed at near atomic resolution—four angstroms—VP7 proteins encircling the spike-shaped VP4 proteins in an interlinked web across the surface of the virus.

Harrison, Grigorieff, and colleagues published the structure in the June 30, 2009, issue of *Proceedings of the National Academy*

of Sciences. The VP7 proteins appear to hold VP4 in place until conditions—probably a drop in calcium concentration in the host cell vesicle surrounding the engulfed virus particle—prime the particle for infection. The VP7 proteins are then released, freeing VP4 to puncture the membrane of a target cell. The antibody crystallized with VP7 apparently prevents infection by clamping VP7 onto the virus particle and preventing VP4 from puncturing the cellular membrane.

Harrison now wishes to understand how, and where within the cell, the spike protein VP4 carries out this puncturing step. He plans to investigate it with x-ray crystallography, with cryo-EM, and using light microscopy to image virus particles entering living cells. “We’re now poised to ask, how does VP4 actually do it?” he says. “We don’t know the answer yet, but we know a lot about what it must involve, and we know the kinds of experiments we need to do next.” ■ —SARAH GOFORTH