Murder, Suicide, and a Trip to Princeton

Or how a virus can cause a cell to destroy itself, and how a meeting at Princeton University contributes to research at the NSLS

Viruses use actin, the most abundant protein in the cells that they are infecting, to break open the cells to allow new viruses to escape and infect others.

This discovery, described in the November 29, 2002, *Journal of Biological Chemistry*, was made by Walter Mangel, Biology Department, and his team, in collaboration with the laboratories of Nancy Reich, Stony Brook University, and Gerard Marriott, University of Wisconsin. The research is funded by the Office of Biological & Environmental Research in DOE's Office of Science and the National Institutes of Health (NIH).

The findings, which build upon Mangel's earlier research on how virus particles become infectious, may lead to better antiviral remedies.

Self-portrait by Wally Mangel, Biology Department, in graphite. The bar graph (upper left) shows the results of an experiment by Bill McGrath, Biology, in Mangel's lab, that was published in Nature. This was the first description of the two viral cofactors that stimulated the protease. Now, Mangel and his team have identified a third cofactor, the most abundant cellular protein, actin.

Murderous Intent

When a virus infects a cell, one of its objectives is to make new virus particles that can infect and reproduce in adjacent cells. One virus can produce thousands of virus particles within one cell.

The virus's next step is to cause the cell to lyse, or self-destruct, by breaking open and releasing newly synthesized virus particles that can infect adjacent cells. Different viruses employ different strategies in lysing cells late in infection. If cells with newly synthesized virus particles do not break open, then the virus infection is essentially aborted.

Hatchet-Job Enzymes

Mangel's group has been studying a protease made by human adenovirus, a virus that causes gastrointestinal and respiratory infections, and conjunctivitis. A protease is an enzyme that cleaves, or cuts, other proteins, making them shorter.

In human adenovirus infection, newly synthesized virus particles are not infectious. They contain precursor proteins, which are slightly larger than those that are seen in infectious virus. The precursor proteins act as "construction" parts, which are needed for virus particle assembly.

Mangel's group had shown that the adenovirus protease is synthesized in the cytoplasm of cells — the area between a cell's membrane and nucleus — in an inactive form. The protease migrates from the cytoplasm into the nucleus where it is incorporated into newly synthesized virus particles. Inside these particles, the protease activates by binding to the viral DNA and cleaving off a small fragment, pVIc, of a viral protein. The pVIc then binds to the protease and fully activates it.

Said Mangel, "Builders remove supportive scaffolding after completing a construction project. Similarly, the activated protease cleaves the viral precursor proteins, leaving infectious virus particles behind. Both the viral DNA and pVIc are cofactors for the adenovirus protease's becoming active in that their presence enhances its cleaving ability."

The Princeton Connection

A connection between cell lysis and the adenovirus protease was made serendipitously when Mangel gave a seminar on his protease research at Princeton University. He was invited by Clarence Schutt of the Chemistry Department, whom he had met while serving on a special study section at the NIH. During the talk, after Mangel showed the sequence, or formation, of pVIc, Schutt said that he had seen that sequence elsewhere.

"I found this hard to believe," Mangel admitted. "Ever since we'd discovered pVIc, our group had periodically searched the various databases to see if there were other proteins that contain the pVIc sequence — and we never found anything."

Later that day, Schutt showed Mangel the sequence of actin. Mangel saw that, of the last seven amino acids of actin, four are identical and three homologous to the sequence of pVIc.

At first, Mangel said, he was quite shocked by this revelation, but then he began to think of its implications.

"Wow! Can you spare some actin for me to take back to BNL tomorrow?" he asked. The next day, Mangel brought the actin to his lab, where Diana Toledo, formerly of Biology, was waiting. They tested to see if actin could be a cofactor.

"In the very first experiment, it was clear that actin can be a cofactor for the adenovirus protease," Mangel said. "Incubating actin and the adenovirus protease increases the cleaving activity of the adenovirus protease, just as pVIc can do."

Cell Suicide

Actin is the most abundant protein in a cell. Mangel explained that one of its functions is to form actin polymers that act as the steel girders in skyscrapers, giving rise to the structure of a cell. Conversely, if their actin is destroyed, cells lose their shape and eventually break open.

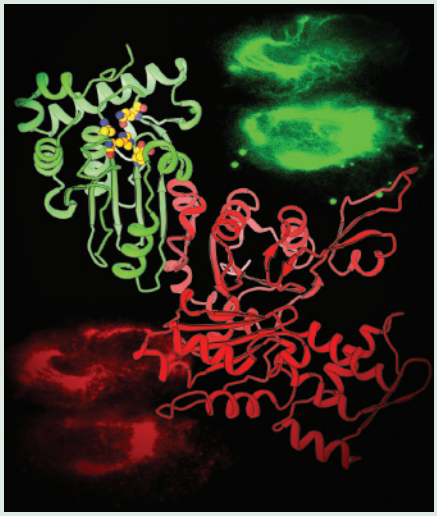
In the November 29 article, the authors show that the adenovirus protease binds to actin, and thus becomes activated. They also noted that the sequence of actin contains two sites that can be cleaved by the active adenovirus protease. Incubation of actin with the adenovirus protease not only activates the protease, but also allows it to cleave actin at those two sites.

"Thus, actin is a cofactor for its own destruction, a new and philosophically interesting way for a virus to lyse cells," Mangel commented.

Crystallizing the Future

Mangel's group has already set up crystallization trials of actin bound to the adenovirus protease. "If we can crystallize the complex, then we may be able to determine its atomic structure at the National Synchrotron Light Source," Mangel said. "That structure would then be used to find drugs to prevent the interaction between actin and the adenovirus protease. Such drugs could serve as a new type of antiviral agent."

—Karen McNulty Walsh



Predicted structure of the adenovirus proteinase (AVP)-actin complex and colocalization within cells of AVP and cytokeratin-18. The crystal structure of AVP (green) was docked onto a portion (blue) of the crystal structure of actin (red). The red green and yellow balls on AVP are the atoms involved in catalysis. In cells containing an AVP-green fluorescent protein complex, its fluorescence (green) colocalizes with the fluorescence (red) from an anti-cytokeratin-18 antibody, consistent with the cleavage of cytokeratin-18 by an AVP-actin complex.