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## Researchers Determine 3D Structure of Key Bacterial Protein

Researchers from the Howard Hughes Medical Institute (HHMI) have determined the three-dimensional structure of invasin, a protein that allows a close relative of the bubonic plague bacterium to infect intestinal cells and cause food poisoning.

When the bacterium *Yersinia pseudotuberculosis* is consumed in contaminated food, it gains access to certain cells in the intestinal epithelium called M cells. Once inside the intestines, *Y. pseudotuberculosis*, which is related to the organism that causes bubonic plague, binds to receptors on M cells. This leads to the entry of the bacteria into regional lymph nodes in the intestine. The growth of bacterial colonies in the lymph nodes triggers intense nausea, vomiting and diarrhea. It turns out that invasin is crucial to the bacterium's ability to establish a foothold in the lymph nodes, says Ralph Isberg, an HHMI investigator at Tufts University School of Medicine.

When Isberg first began studying *Y. pseudotuberculosis* in the 1980s, "there was very little known about how any bacterium was able to enter into an animal cell and trigger infection," he said.

To better understand how *Y. pseudotuberculosis* causes illness, Isberg set about cloning genes from *Y. pseudotuberculosis* and inserting them one by one into a second bacterium used frequently in laboratory research. He realized he had made an important discovery when a strain of the laboratory bacterium harboring a single *Y. pseudotuberculosis* gene had acquired the ability to enter animal cells. "This single gene (*inv*), and the invasin protein it coded for, was sufficient for infection of cells in culture," Isberg said.

Researchers now know that the aptly named bacterial protein binds more tightly to integrins the receptors for invasin than do other proteins, or ligands. To learn how invasin out-competes the natural ligands for their place on the receptors, Isberg and HHMI research assistant Michele Brown collaborated with HHMI structural biologist Pamela Bjorkman and graduate student Zsuzsa Hamburger, both of whom are at the California Institute of Technology. Bjorkman and Hamburger crystallized the invasin protein and

used x-ray diffraction techniques to determine its three-dimensional structure. The researchers published their work in the October 8, 1999, issue of the journal *Science* .

The three-dimensional structure reveals that invasin is a rod-like protein resembling five tandemly arranged beads. In comparing invasin to one of the integrins' natural ligands, called fibronectins, Bjorkman and her colleagues found that the two types of proteins share some structural features, such as an extended structure and a similar distribution of key binding residues, but are otherwise quite different in their folding topologies.

"It seems that the bacteria got the essence of what's needed to bind to an integrin, but they did it in their own way," Bjorkman said. "Invasin basically distilled out the important features for integrin binding, then put them into a different folding context."

There are antibiotics available that can kill *Y. pseudotuberculosis* , but Bjorkman and Isberg say that structural information about invasin, and proteins from other types of bacteria that are "modeled on" invasin, may lead to better antibacterial agents. "We now have a more specific target," said Isberg.

A drug that blocks binding to crucial regions of the invasin receptors should be able to prevent the bacterium from entering a cell, Isberg said. Also, knowing where both invasin and fibronectin bind to integrins gives scientists a new tool to explore ways of altering integrin function.

With the structure of invasin solved, Isberg and his colleagues plan to look more closely at the messages the cell receives when invasin binds to its integrin receptors. They are also interested in the process by which this binding triggers uptake of the bacteria by epithelial cells in the intestine.

Meanwhile, the next step for Bjorkman and her colleagues is to determine the structure of invasin and an integrin bound together. Both efforts should offer additional insight into the way cells talk to each other and the way bacteria cause disease.

Bjorkman believes that similarities in the structures of invasin and fibronectin suggest the bacterial and host proteins bind to integrins in a similar way. "Choosing an integrin as a receptor and copying the way a host protein binds to it ensures that the bacteria can infect its host," Bjorkman said. "Host cells need integrins, so they cannot be changed to avoid binding invasin since that would disrupt important host functions."