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How *E. coli* Bacteria Put a Death Grip on Intestinal Cells

A team of molecular biologists and x-ray crystallographers has produced the first detailed picture showing how pathogenic *E. coli* bacteria achieve a molecular death grip on intestinal cells. The structure may reveal critical steps in *E. coli* attachment that scientists can attack with drugs designed to thwart infection by halting bacterial binding.

The *E. coli* bacteria include the enteropathogenic strains that are a major cause of childhood diarrhea in developing countries, killing close to one million children each year due to dehydration and other complications. This family of pathogens also includes *E. coli* O157:H7, a closely related strain that contaminates hamburger and other foods. *E. coli* O157:H7 causes at least 20,000 cases of bloody diarrhea and more than 200 deaths annually in the United States due to kidney failure, especially in young children and the elderly.

Natalie C. J. Strynadka and B. Brett Finlay at the University of British Columbia led the collaborative team of researchers that solved the structure of the *E. coli* intimin-receptor complex. The researchers reported their findings in an article in the June 29, 2000, issue of *Nature*. Finlay is an HHMI international research scholar in Canada. Yu Luo, a postdoctoral fellow in the Strynadka laboratory, was first author of the report.

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Intimin is a protein, called an adhesin, that is anchored in the bacterial outer membrane. The bacterium attaches itself to a target intestinal cell by first "harpooning" and embedding its receptor, called translocated intimin receptor (Tir), in the epithelial membrane of the host cell. The new structural information shows how the bacterium joins to intestinal cells by attaching its intimin proteins to the inserted Tir proteins.

"We knew that these molecules were involved in attachment and that this binding was essential for disease, but we had no idea at the molecular level how they were actually linking up," said Finlay.

The researchers obtained the picture of the intimin-Tir complex using x-ray crystallography. In this widely used technique, x-rays are beamed through crystals of a protein and the intricate patterns of diffracted x-rays that emerge are analyzed using a computer to deduce the protein's structure.

In the case of the intimin-Tir complex, however, the crystals of the full-size protein complex were far too unstable to be analyzed.

"We approached solving the complex of these two membrane proteins by creating several dozen constructs to try to get down to the smallest functional fragments from each of the proteins," said Strynadka. "Once we had these, we were able to produce crystals for analysis."

With postdoctoral fellow Elizabeth Frey creating the protein fragments and postdoctoral fellow Luo of Strynadka's laboratory performing the crystallographic analysis, the scientists finally formed a picture of the entire intimin-Tir complex.

The structure reveals that the protein-protein complex features rigid "arms" with attaching "hands" on the end of the intimin that reach out to grasp the receptor. The protein arms are bent so that they can clasp the bacterium closely to the surface of the intestinal cell.

"It's the first time anyone has ever solved the structure of a bacterial adhesin complex with its receptor," said Finlay. "And in this case, it's a neat twist because the receptor is actually a bacterial protein that *E. coli* harpoons into the mammalian cell."

A particularly important finding, said Finlay, is that the crystallographic structure shows that each Tir consists of two joined units, called a dimer.

"That dimerization crosslinks the system," he said. "It's like Velcro surfaces sticking to one another. When you just have one Velcro link, it doesn't stick very well, but if you have a whole mat of them you get very strong adhesion." In attaching to the intestinal cell, the bacterium likely uses myriad intimin-Tir links, said Finlay.

"It's a big bacterium, and like the Hindenburg docking, one rope is not going to tie it down," he said. "But when you have many of these complexes latching the bacterium onto the surface, you get a strong adherence because these things are tough."

Also surprising, he said, was that the structure of the intimin protein -- which attaches to a receptor that the bacterium shoots into its host cell -- closely resembles that of another bacterial attachment protein called invasin, which is found in a different bacterial pathogen. The invasin protein, in sharp contrast, attaches itself to a receptor found naturally in the membrane of its mammalian host cell.

"It was a surprise that these two bacterial molecules that attached to different kinds of receptors seemed to look very similar," he said. Further study of these intimin-Tir interactions could yield important fundamental insights into how other types of bacteria attach to cells, and how cells in general attach to one another, said Finlay and Strynadka. Finlay also emphasized that the intimin-Tir structure will offer valuable insights to aid drug design.

"Once you know the molecular structure of this complex, you can think of doing rational drug design of inhibitors," said Finlay. "By knowing the sequence structure of the proteins involved, you know which parts are absolutely key for adherence, and you can design drugs to thwart that attachment." According to Finlay, such structural information could lead to the development of a childhood vaccine to prevent *E. coli* infection. Since *E. coli* infection can be passed from cattle to humans, the researchers are also working on developing a similar vaccine for cattle, he said.