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The Ties that Bind

Deep within certain types of *Escherichia coli* is a package of genes that are always on red alert, ready to attack when the bacterium collides with a host cell to its liking.

A master at adhering to mammalian intestinal cells, pathogenic *E. coli*'s assault is efficient and precise: Tiny tethers radiating from *E. coli* hold the target mammalian cell in a firm embrace. Next, a syringe-like pump emerges from within the bacterium to administer a bolus of bacterial proteins that disrupts the target cells' intracellular signaling. This, in turn, perturbs the regular meshwork of structural proteins that support host cells. As the bacterial proteins are pumped into the host cells, a pedestal forms underneath the bacterium and raises it mightily above the conquered mammalian cells. The instructions to carry out all of these activities are hard wired into the bacterium's genome.

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Researchers are now learning the intimate details of how this potentially deadly family of bacteria bind to target cells with preternatural affinity. Just a few years ago, however, scientists did not have such a complete picture of the molecular details of enteropathogenic *E. coli* (EPEC), the bacterium responsible for causing severe diarrhea and a large number of deaths among infants in developing nations. Now this ancient microbial marauder is finally yielding its secrets to the persistent inquiries of microbiologists around the world.

One of those leading the assault is HHMI international research scholar B. Brett Finlay of the University of British Columbia. A two-time HHMI grant recipient, Finlay's explorations of *E. coli* have taken him to the moments of first contact—when the bacterium "sees" its target and "decides" to adhere to it.

For years Finlay and his team have chased down various leads that they hoped would reveal the tools that the bacteria deploy when binding to human cells. They believe that if bacterial adhesion is understood in finer detail, scientists can design more effective agents to prevent bacterial infection.

Much of their work has focused on finding the various receptors that the bacteria use to grab hold of host cells. Finlay's group and others are pursuing a number of different strategies to learn more about the early stages of bacterial infection. Along the way, they are learning a great deal about the likes and dislikes of *E. coli*, its structural proteins and the organization of its genome.

Their studies of EPEC revealed one mysterious protein, Hp90. It was called Hp90 because it behaves as a host protein that weighs 90 kilodaltons. Hp90 is tyrosine phosphorylated in host cells and is also the receptor that EPEC uses to bind to host cells.

Hp90 came to light as Finlay's team tried to figure out why EPEC would adhere to host cells only if those cells were previously infected with other bacteria. It seemed that previous bacterial infection somehow primed Hp90 in the host cell membrane so that EPEC could then attach to Hp90. The observation didn't make sense, but Finlay's group attempted an explanation: "We immediately thought this was an interesting concept—the first bacteria were basically warming up the host cells so that EPEC could grab onto those cells," he said. "The next big question was, 'What is this 90-kilodalton protein doing in host cells?'"

No one had been able to isolate Hp90, and the last thing Finlay wanted to do was lose precious time chasing a phantom host membrane protein that may or may not be involved in EPEC adherence. "We're microbiologists, and it wasn't our idea of fun to isolate proteins from mammalian cell membranes—that's a whole different field," he said.

Still, they persevered and began the search for Hp90. At the same time, Brendan Kenny, a postdoctoral fellow in Finlay's lab who is now on the faculty at the University of Bristol in England, started studying the host-bacteria interaction in greater detail, looking to see if the bacteria squirted any proteins into the host cell.

Kenny labeled EPEC with radioactive chemicals that would act as tracers so he could follow the trajectory of bacterial proteins once they contacted host cells. "Brendan saw a few proteins going into the host cell," Finlay said, "and one of them looked very much like Hp90."

For a moment, Finlay's group entertained the idea that Hp90 was of bacterial origin. "The idea seemed so radical that we immediately wanted to dismiss it," he said. "But at the same time we also said that we better chase it down." One of the emerging paradigms in microbiology is that the truly "bad proteins" that do all of the bacterium's dirty work are secreted proteins. These proteins are injected into the host cell or into the extracellular milieu to make conditions more favorable for invasion. In this context, Finlay said, it was

possible that Hp90 could be a secreted protein. One problem with this line of reasoning, Finlay knew, is that EPEC does not have any secreted proteins that weigh around 90 kilodaltons.

To their surprise, however, they did find that under special conditions EPEC does secrete a 78-kilodalton protein. "We raised antibodies to the 78-kilodalton protein, and almost on a lark we asked if those antibodies would also bind to the 90-kilodalton protein," Finlay explained. When the antibodies bound to the 90-kilodalton protein, Finlay knew the group had found something special: it appeared that the 78-kilodalton protein and 90-kilodalton protein were similar if not the same protein.

One gnawing question seemed to contradict the idea: How could the proteins be the same if they had different molecular weights? They knew that some type of protein modification could account for the extra weight. "We thought that maybe the weight difference was due to the fact that the bacterial protein goes into the host cell and is phosphorylated," Finlay said. So, they dephosphorylated the 90-kilodalton protein and showed that it was indeed the 78-kilodalton bacterial protein.

When they realized the full implication of the results, Finlay admits, members of the lab were shocked, "At first we were flabbergasted," he said. "We didn't want to believe it because whenever you find something new, unless you can prove it thoroughly it might as well be forgotten."

The team's work, which was published in the November 14, 1997, issue of *Cell*, shows that Hp90 was never a mammalian protein at all, but a bacterial protein. Scientists scouring mammalian cell membranes had been looking in the wrong places all those years. "This has taken everyone aback because we're saying that EPEC encodes its own receptor," Finlay said. "These bacteria carry around their own receptor, which they shoot into host cells and bind to. Every pathogen identified previously was an existing protein on host cells. This appears to be a completely new paradigm."

Finlay is quick to point out that much of what he and others have discovered about EPEC also holds true for its notorious cousin EHEC, enterohemorrhagic *E. coli*, the bacterium that can be found in tainted apple juice and hamburgers. As few as 10 bacteria from an undercooked hamburger are enough to cause the devastating illness that Finlay diplomatically refers to as "hamburger disease." This infection might also lead to the deadly hemolytic uremic syndrome.

This spring Finlay's lab plans to see if the newfound Hp90 receptor—which was renamed Tir—might be useful as a vaccine to prevent EHEC from colonizing newborn calves. With the support of Canada's Beef Development Industry Fund, Finlay hopes to inoculate a small group of calves with the receptor to see if they produce antibodies and prevent EHEC colonization, which is responsible for contaminating beef.

"We don't know if this will work," he said. "But because it's a bacterial receptor, we don't have to worry about blocking the function of a normal host

protein. The results should be interesting."