PROFILE

Profile of Scott J. Hultgren

Nicholette Zeliadt
Science Writer

During her lifetime, an adult woman has a 50% chance of developing a urinary tract infection—one of the most common types of infections (1). Most urinary tract infections are caused by strains of the bacterium *Escherichia coli*, and, for the most part, are easily treated with antibiotics. However, many of these infections become chronic or recurrent because of increasing antibiotic resistance and a variety of other pathogen- and host-related factors, leaving few treatment options. Scott J. Hultgren, a professor of molecular microbiology and director of the Center for Women's Infectious Diseases Research at Washington University in St. Louis, investigates the molecular mechanisms by which bacteria establish infections in the urinary tract and evade the body's innate defenses. Hultgren, a 2011 member of the National Academy of Sciences, has uncovered many of the factors that determine the onset and severity of these infections, revealing promising therapeutic targets in the process. His work may help to change how urinary tract infections are diagnosed, treated, and prevented.

From Chemistry to Microbiology

Throughout Hultgren's childhood, science figured prominently as a regular topic of his family's dinner table conversations. His father was a high school chemistry teacher in Michigan City, Indiana, a small town on the southern tip of Lake Michigan, where Hultgren was born and raised. "He had a way to connect with students," Hultgren recalls, "and always got them interested, asking inquisitive questions." That passion for chemistry also rubbed off on Hultgren, who majored in the subject when he enrolled as an undergraduate at Indiana University in 1977.

However, Hultgren says he instantly fell in love with microbiology when he took a course in environmental microbiology, taught by microbiologist Walter Konetzka, "another tremendous teacher who really inspired me." Although Hultgren ultimately switched his major to microbiology, he found a way to blend his interests while working on a class assignment. "We had to come up with a project and then present it in front of the class after we'd developed it," Hultgren recalls. Most students did something traditional, he says, such as culture bacteria on agar plates; Hultgren decided to create a microbial battery. After consulting with his father and Indiana University professor Howard Gest, an expert on photosynthesis, Hultgren designed his battery using the photosynthetic bacterium *Rhodobacter capsulatus*. Upon exposure to light, the bacterium produces hydrogen gas when cultured in media deprived of nitrogen. In Hultgren's system, a platinum electrode split the gas into hydrogen ions and electrons; the electrons then flowed across a salt bridge to the cathode, illuminating a small light bulb. "That went over pretty big," Hultgren recalls with a chuckle. "The local newspaper ended up writing about it."

After graduating in 1981, Hultgren accepted a position in Denise Hidvegi's laboratory in the Department of Pathology at Northwestern University in Chicago, Illinois. Hidvegi was using light microscopy to look for cancer cells in samples of cervical cells from patients and wanted to be able to later examine the cells by electron microscopy. Hultgren developed a technique for preparing the specimens for light microscopy that preserved the cells' structures when they were subsequently removed from the microscope slides and processed for electron microscopy. These findings formed the basis for his first scientific publication (2).

Stuck on Microbes

In 1982, Hultgren went to work for Anthony Schaeffer, a urologist at Northwestern who was exploring how *E. coli* adhere to the urinary tract epithelium, crucial for the development of a urinary tract infection. Schaeffer had recently found evidence suggesting that adherence was mediated by the binding between pili—long, hair-like protein appendages found on the surface of *E. coli* and other Gram-negative bacteria—and sugar-containing molecules on the surface of urinary tract epithelial cells (3). Hultgren began investigating the role of pili in urinary tract infections in mice—work that he continued in the laboratory of James Duncan, one of Schaeffer's long-time collaborators—as he pursued his doctorate.

Near the end of his doctoral training, Hultgren attended a Gordon Conference on microbial toxins and pathogenesis, where he met Staffan Normark, a leading figure in the field of bacterial pathogenesis. Normark had discovered that the so-called P pilus, produced by strains of *E. coli* that cause kidney infections, contained a specialized adhesive protein known as the PapG adhesin that mediates binding of the bacteria to host cells (4). Through his doctoral research, Hultgren had become interested in studying the molecular basis of pili formation and function, so he applied to become a postdoctoral fellow in Normark's laboratory, then located at the University of Umeå in Umeå, Sweden, where Hultgren's son, Nils, was soon born.

Scott J. Hultgren. Image courtesy of Robert Boston, Washington University School of Medicine.

When Hultgren began his postdoctoral research in 1987, the proteins comprising the P pilus were known: the shaft consisted of repeating units of the protein PapA, a second protein anchored the base of the pilus to the cell membrane, and several others, including the PapG adhesin, were located exclusively at the tip. However, how these proteins were assembled in this precise arrangement to form the pilus remained unclear. As a step toward understanding pilus formation, Hultgren tried to isolate the PapG adhesin,

This is a Profile of a recently elected member of the National Academy of Sciences to accompany the member's Inaugural Article, 10.1073/pnas.1315203110.

so that he could analyze how it interacted with other proteins before it became incorporated into a mature pilus. To Hultgren's surprise, the adhesin purified in a complex with another protein called PapD—an interaction that turned out to be essential for the incorporation of the adhesin into the pilus (5). Soon, it became clear that PapD also interacted with each of the proteins destined for incorporation into the pilus. Hultgren nicknamed PapD "the chaperone," reflecting its crucial role in folding, stabilizing, and transporting the pilus proteins to the membrane, where they became assembled into mature pili. "That was a key discovery," Hultgren says. "It's interesting how much of my work can be traced back to that complex."

From Chaperone to Usher

In 1989, Normark was recruited to become the chair of the Department of Microbiology at Washington University, and Hultgren was the first professor he hired. Hultgren continued to study the mechanisms of pilus formation and soon uncovered the function of yet another component of the pilus assembly apparatus: PapC, which he dubbed "the usher." He found that this integral membrane protein received the pilus proteins transported by the chaperone and ushered them into the growing pilus in the appropriate order (6). He named this choreographed system of pilus formation the chaperone–usher pathway. This pathway is now known to have hundreds of homologous systems in diverse Gram-negative bacteria.

Hultgren's laboratory has continued to reveal important insights into the structural basis of P pilus formation, as well as the assembly of an analogous adhesive fiber on urinary tract-infecting *E. coli*, the type 1 pilus. For his discoveries, Hultgren received the 1998 Eli Lilly and Company–Elanco Research Award from the American Society of Microbiology—the association's oldest and most prestigious prize, which recognizes top microbiologists under the age of 45 (when Hultgren received the award, it was given to investigators younger than 40).

In the mid-1990s, Hultgren began to turn his attention toward understanding how those adhesive fibers enable the bacteria to gain a foothold in the urinary tract. He found that binding of type 1 pili to bladder epithelial cells promotes their invasion into those cells. Once inside and sheltered from urine flow, the immune system, antibiotic treatments, and other antimicrobial factors, the bacteria rapidly multiply and aggregate into biofilm-like masses Hultgren calls intracellular bacterial communities (7). However, signaling within the infected host cells causes those cells to die and be shed into the urine (8). "It's a mechanism that the bladder presumably uses to try to get rid of the infected cells," Hultgren explains.

At that point, the bacteria "are sort of like salmon swimming upstream," he says. "They need to get into cells and then get out before those cells die and slough off."

In addition, Hultgren found that the bacteria can evade this host defense mechanism by invading bladder cells deep below the surface—a process that is also mediated by type 1 pili. Subsequent studies in his laboratory revealed that the intracellular bacterial communities are transient in nature: they form and then later disperse, the bacteria escaping their temporary safe haven and spreading into neighboring cells, where they can repeat the process all over again. "This is the way that *E. coli* gains a foothold in the bladder at very acute stages of the infection," Hultgren says. The findings revealed that urinary tract infections were more complex than previously thought. "We're just beginning to dissect the complexities that occur at the host-pathogen interface," Hultgren says. "The more we learn about this disease, the more we realize we don't know."

Changing Clinical Paradigms

Hultgren hopes that by understanding the molecular crosstalk at the host–pathogen interface, his laboratory can come up with therapeutics that might hold promise in treating or preventing urinary tract infections, particularly the chronic, recurrent infections where antibiotic therapies fall short. Hultgren has been evaluating compounds called pilicides, developed by the chemist Fredrik Almqvist at Umeå University, that bind the chaperone and interfere with the assembly of pili (9). Hultgren has also been working with James Janetka, a chemist at Washington University, who has developed compounds that bind to the adhesin on the type 1 pilus and block the initial attachment of *E. coli* to the urinary tract epithelium (10). Hultgren recently showed that orally administered adhesin inhibitors are effective in treating and preventing experimental urinary tract infections in mice (11). Hultgren says he's hopeful that these compounds, if brought to the clinic, could be promising therapeutics for individuals with chronic or antibiotic resistant infections, and may also help to reduce the use of antibiotics and the emergence of drug-resistant strains.

However, what factors determine whether an individual will develop an acute, self-limiting urinary tract infection versus a chronic or recurrent infection? Hultgren has found that the fate of the disease may be determined early in the infection. Using a mouse model of urinary tract infection, Hultgren discovered that the animals that develop chronic infections produce a set of biomarkers within 24 h of infection, suggesting that there may be what he calls a host–pathogen checkpoint that, when triggered, determines disease outcome (12). If the checkpoint is not triggered and the biomarkers do not appear, then the infection is cleared. Furthermore, the mice that developed a chronic infection were more prone to a subsequent infection compared with those that had initially experienced a self-limiting infection, suggesting that the checkpoint may also determine susceptibility to recurrent infection. Hultgren says that if these results can be applied to humans, they could explain why some individuals are more prone to developing chronic or recurrent urinary tract infections than others.

Unified Research Front

Hultgren's Inaugural Article (13) unites the two conceptual frameworks that have defined his career thus far: the mechanisms of pilus biogenesis and the molecular determinants of urinary tract infection severity. He has discovered that the adhesin located on the tip of the type 1 pilus—the protein that mediates bacterial binding to the urinary tract epithelium and invasion of host cells—may exist in a dynamic equilibrium between a binding and a nonbinding conformation. Although he does not yet fully understand why the protein needs to be in this equilibrium, it seems to be important for pathogenesis, because genetic variations favoring one conformation or the other lock the adhesin in its binding conformation and attenuate the bacteria. In effect, the conformation of the adhesin regulates bacterial binding and invasion, which determines whether the host pathogen checkpoint is triggered and influences the outcome of the disease, he says.

In the near future, Hultgren will continue to probe the basis of recurrent, chronic infections, and hopes to better understand the complexities of the host pathogen checkpoint. He says experiments that could reveal more about the genetic programs bacteria use in their interactions with the host, how those interactions influence signaling cascades within host cells, and the precise signals that trigger the host pathogen checkpoint are forthcoming. He also wants to identify genetic factors in humans that may predispose individuals to severe forms of urinary tract infections. "I feel that UTI is one of the diseases that we are close to getting a handle on—we really could apply the basic research

in practical applications," Hultgren says. "And I'm really excited about that opportunity. The more we understand, the more confident I feel that we'll be able to come up with a clinically successful therapeutic one day."

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