STORY OF DISCOVERY

Bacterial Pili—Molecular Initiators of Bladder and Kidney Infections

It all starts with a "handshake" between two molecules of almost infinitesimally small size: a tiny, hairlike projection from the surface of a bacterial cell and a small cluster of sugar molecules on the surface of a human epithelial cell. From this molecular interaction arise over eight million doctor visits each year. A urinary tract infection (UTI)—which may involve the bladder, kidneys, or both—begins when a molecule on the surface of a bacterial cell recognizes and binds to a molecule on the surface of its target urinary tract epithelial cell. Bladder and kidney infections are important public health concerns because many individuals suffer recurrent infections. Because of the wide prevalence of UTIs, their tendency to recur, and the potential for bladder infections to progress to more serious kidney infections, researchers have long sought to understand the first critical steps in infection. The identification and characterization of the mechanism by which bacteria adhere to epithelial cells is the key to designing therapies to block this process and prevent these infections. Recently, research from the field of structural biology—the study of how molecules interact with one another—has yielded important insights into the earliest stages of UTIs and provided fresh evidence that strategies to block the attachment of bacteria may represent an effective way of treating the initial infection as well as a viable approach to preventing their recurrence.

The adhesion of bacteria to the cells of the urinary tract is mediated by proteins present on the surface of the bacterial cells. Over twenty years ago, studies implicated these proteins in this process. Since that time, families of proteins have been discovered, the genes that encode them have been cloned, and they have been the subject of much research. Although the genetics of these bacteria are well-characterized, studies of bacterial genetics illustrate a paradox about modern molecular biology: as more is learned about the genes of organisms, the more researchers appreciate that genes tell only part of the story. A gene represents a set of instructions for assembling a chain of amino acids in a specific sequence to form a protein. In order for this to occur, a gene must first be turned "on" so that the information within the gene can be read by the cell. This blueprint must then be faithfully translated into a mature protein. However, functional proteins do not exist as linear chains of amino acids; rather, they fold back upon themselves, assuming intricate shapes with highly complex topography. To truly understand how a protein functions, and how it interacts with other proteins, it is often necessary to understand how these proteins are assembled from their genetic instructions, how this process is regulated, and what form these proteins ultimately assume.

Among the people looking for this knowledge are structural biologists—scientists interested in how molecules come together to form critical components of cells. These researchers have long studied protein assembly using bacteria as a model system. Pili are tiny rod-shaped projections from the surface of many bacteria that can act as adhesion molecules, facilitating cell-to-cell contact and communication. Structural biologists have determined that these hairlike pili are formed through a complex series of interactions that begin within assembly of the pilus base within the bacterial cell and the transport of the elongated pilus across the outer cell membrane. As a model system to study protein assembly, pili have proven to be a very rich source of information about the mechanics of protein assembly and transport within the cell. But pili are important for reasons beyond their value as models of protein assembly.

In UTIs, attachment of bacteria to the surface of the epithelial cells that line the urinary tract is a key event, and a better understanding of this process might reveal novel approaches to preventing infection. Not surprisingly, this initial interaction is mediated in large part through proteins on the surface of the bacterium that

recognize and bind specifically to molecules on the surface of the epithelial cells—including pili. Pili consist of multiple protein subunits each encoded by a specific gene. It has long been known that the presence of certain kinds of pili on a cell's surface increases the number of *E. coli* capable of infecting the urinary tract and enhances the persistence of the infection. Although all strains of uropathogenic *E. coli* possess the genes necessary to give rise to pili, the expression of a particularly critical one is controlled by a small segment of DNA located nearby. This small invertible element is capable of "flipping" its relative orientation within the chromosome and thereby controlling gene expression: when it faces one direction, expression of the gene is turned "on" and pili are present on the cell surface; conversely, when the element faces the opposite direction, the gene is turned "off" and the protein is absent. Relatively little has been known about why this element flips, how its orientation might change during an actual infection, and what the implications of these changes could be.

To answer these questions, scientists recently isolated different strains of *E. coli* from cystitis—bladder infection—or pyelonephritis—kidney infection—and then examined the ability of the bacteria to change the orientation of this invertible element. After culturing both bacterial strains under conditions that maintained the element in the "off" position, the strains were introduced into mice. Subsequently, the bladders were removed and the orientation of the invertible element within the bacteria analyzed. The element in the bacterial strain that was originally isolated from the cystitic strain quickly reverted to the "on" orientation; in contrast, it remained largely "off" in bacteria from the pyelonephritis-causing strain. This observation suggests that cystitis-causing strains have an enhanced ability, relative to pyelonephritis-causing strains, to change the orientation of the invertible element to the "on" position and thereby alter pilus gene expression. This finding suggests that bacteria that infect the bladder may rely more heavily on pilus-mediated attachment during infection than bacteria that infect the kidney. It also illustrates how changes in expression of a particular gene—and not its presence or absence—can influence the site of infection in UTIs.

Once bacteria find themselves in the kidney, pili again play a role in adhesion and infection. A protein that sits atop the pilus—PapG—is involved in recognizing a specific molecule on the surface of the kidney cell and thereby mediating binding of the bacteria to the kidney cell. PapG's partner in this molecular interaction is a group of sugar residues on the outer membrane of the human kidney cell—globoside. Scientists have deduced the three-dimensional structure of PapG alone and in a complex with globoside. This detailed molecular snapshot—with a resolution finer than one-millionth of a meter—has allowed researchers to identify which areas of the PapG protein mediate binding of the bacterium to the host cells, as well as to pinpoint which regions of the host globoside are important for this interaction. By defining the specific molecular interaction necessary for binding to occur, this insight not only sheds light on a key event in infection but may also lead to the development of vaccines that target the disease process at its earliest stages.

Even with all of these insights into the molecular events that underlie the earliest stages of infection, the sad fact remains that, after a successful course of antibiotic therapy, a significant number of women will suffer a relapse of their UTI. Why this is so has remained a mystery for many years. New research has provided fresh insights into why many bladder infections recur—and once again highlights the role of pili. It seems that bacteria use their pili not only to recognize and bind to bladder cells during the initial infection but also to get inside of these cells. Invasion of these bladder cells may provide the bacteria with a relatively safe environment in which they may either replicate or go into "hibernation," only to emerge later—and cause a subsequent UTI. According to this model, following the initial UTI the bacteria persist at low levels, continuously invading a small number of cells, escaping, and re-infecting neighboring cells. Insights into the role of pili in this process might allow the design of therapies designed to interrupt this cycle of infection and relieve the burden of recurrent UTIs.

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The study of pili illustrates how technical and highly specialized research into a seemingly obscure question—how proteins are assembled in bacteria and what shapes they assume—can also have relevance to a common and costly health issue—UTIs. From bacterial models of protein assembly to critical players in infection and possible targets for future therapies, the story of pili shows that there is no such thing as something too small to investigate.