Microreview

Adhesion and entry of uropathogenic *Escherichia coli*

**Matthew A. Mulvey**  
Pathology Department, 5B412 SOM, University of Utah, 30 North 1900 East, Salt Lake City, UT 84132-2501, USA.

**Summary**

To effectively colonize a host animal and cause disease, many bacterial pathogens have evolved the mechanisms needed to invade and persist within host cells and tissues. Recently it was discovered that uropathogenic *Escherichia coli*, the primary causative agent of urinary tract infections, can invade and replicate within uroepithelial cells. This can provide *E. coli* with a survival advantage, allowing the microbes to better resist detection and clearance by both innate and adaptive immune defence mechanisms. Adhesive organelles, including type 1, P, and S pili along with Dr adhesins, promote both bacterial attachment to and invasion of host tissues within the urinary tract. Interactions mediated by these adhesins can also stimulate a number of host responses that can directly influence the outcome of a urinary tract infection.

**Introduction**

Upon entering the urinary tract, uropathogenic bacteria face a multitude of both constitutive and inducible host defences that include the bulk flow of urine, numerous antibacterial molecules, and the influx of effector immune cells. To overcome and evade these defences and persist, uropathogens have evolved a number of mechanisms to both adhere to and invade host tissues. The successful use of these virulence mechanisms is reflected in part by the prevalence of urinary tract infections (UTIs), which are currently among the most common bacterial infections acquired by humans. It is estimated that 11% of women in the United States experience at least one physician-diagnosed UTI per year, and about 60% of women will have one or more UTI during their lifetime (Foxman et al., 1999; Johnson et al., 1998; Zhang et al., 2000). Epidemiological, cell culture and animal studies have indicated that a number of factors encoded by UPEC can modulate bacterial virulence within the urinary tract. These virulence factors are usually encoded on the chromosome of UPEC and are often part of large, unstable chromosomal regions known as pathogenicity islands (Hacker and Kaper, 2000; Muhldorfer et al., 2001). Virulence factors associated with UPEC include the toxins α-haemolysin and cytotoxic necrotizing factor 1 (cnf1), the siderophores aerobactin and enterobactin, lipopolysaccharide (LPS), capsules, and a number of adhesive organelles (Johnson, 1991; Muhldorfer et al., 2001). The presentation of adhesive molecules (adhesins) by UPEC can modulate bacterial virulence within the urinary tract. These virulence factors are usually encoded on the chromosome of UPEC and are often part of large, unstable chromosomal regions known as pathogenicity islands (Hacker and Kaper, 2000; Muhldorfer et al., 2001). Virulence factors associated with UPEC include the toxins α-haemolysin and cytotoxic necrotizing factor 1 (cnf1), the siderophores aerobactin and enterobactin, lipopolysaccharide (LPS), capsules, and a number of adhesive organelles (Johnson, 1991; Muhldorfer et al., 2001). The presentation of adhesive molecules (adhesins) by UPEC, more so than the expression of toxins or other virulence factors, is arguably the most important determinant of pathogenicity. Adhesins enable UPEC to bind to host cells within the urinary tract and avoid rapid clearance with the bulk flow of urine. Adhesins can also contribute to virulence in a number of other ways; directly triggering host and bacterial cell signalling pathways, facilitating the delivery of other bacterial products to host tissues, and promoting bacterial invasion. This review focuses on the mechanisms and because of anatomical differences between the sexes. In otherwise healthy women, most uropathogens originate in the intestinal flora and enter the bladder by an ascending route, via the urethra with an interim phase of periurethral and distal urethral colonization (Sobel, 1997; Muhldorfer et al., 2001). The bladder is the primary site of infection in about 95% of all UTIs. Frequent or urgent voiding and sometimes suprapubic pain characterize bladder infections (cystitis) (Muhldorfer et al., 2001). Infection of the kidney (pyelonephritis) is usually a more serious problem and is associated with flank pain, nausea, vomiting, fever, sweats, malaise, and potentially the symptoms that are characteristic of cystitis. In about 30% of cases pyelonephritis is complicated by bacteraemia which may lead to sepsis. The primary causative agents of UTIs, accounting for greater than 80% of these infections, are strains of uropathogenic *Escherichia coli* (UPEC) (Sadler et al., 1989; Hooton and Stamm, 1997; Svanborg and Godaly, 1997).

UPEC isolates, like enteric *E. coli* pathogens, are a genetically heterogeneous group and can vary significantly in their abilities to colonize and persist within either the bladder or the kidneys (Foxman et al., 1995; Johnson et al., 1998; Zhang et al., 2000).
Table 1. Adhesins commonly associated with UPEC isolates.

| Organelle        | Specific adhesin | Host receptors                                      | Cells recognized                                                                                   | Associated disease                  |
|------------------|------------------|----------------------------------------------------|---------------------------------------------------------------------------------------------------|-------------------------------------|
| Type 1 pili      | FimH             | Mannosylated glycoproteins                         | Bladder and kidney epithelial cells, buccal cells, erythrocytes, mast cells, macrophages, neutrophils, extracellular matrix, other bacteria, implants | Cystitis, sepsis, meningitis        |
| P pili           | PapG(I, II, III)  | GbO3, GbO4, GbO5                                   | Kidney epithelial cells, erythrocytes                                                               | Pyelonephritis                      |
| S/F1C pili       | SfaS, SfaA/FocH   | Sialic acid residues, plasminogen/β-GalNac-1,4-Gal | Bladder and kidney epithelial cells, erythrocytes, endothelial cells                                | Ascending UTIs, sepsis, meningitis |
| Dr adhesins      | Various          | DAF (CD55), CD66e, type IV collagen, α5β1 integrin | Bladder and kidney epithelial cells, erythrocytes, neutrophils, interstitial compartments of kidney | Cystitis, pyelonephritis, diarrhoea, sepsis |

consequences of UPEC adherence and invasion within the urinary tract.

UPEC adhesins

Bacteria assemble adhesins on their surface as monomers, simple oligomers, or components of supramolecular fibers called fimbriae or pili (Hultgren et al., 1996). The adhesive organelles most commonly associated with UPEC include type 1, P, and S/F1C-related pili and the Dr family of adhesins (Johnson, 1991) (Table 1). The assembly of these adhesins, and of a large number of other adhesive organelles expressed by *E. coli* and other species, is mediated by highly conserved periplasmic chaperones and outer membrane usher proteins. The chaperone-usher pathway has been reviewed in detail elsewhere (Hung and Hultgren, 1998; Sauer et al., 2000). Here, the structures and functions of the predominant adhesive organelles associated with UPEC are considered.

Type 1 pili

Of the various adhesins encoded by UPEC, type 1 pili are by far the most prevalent (Brinton, 1959; Buchanan et al., 1985; O’Hanley et al., 1985; Langermann et al., 1997). These organelles are composite fibers varying from a few fractions of a micron to greater than several microns in length (Jones et al., 1995). They consist of a 7 nm thick helical rod, made up of repeating FimA subunits, coupled to a short 3 nm wide tip fibrillum structure containing the adhesin FimH and two adaptor proteins, FimF and FimG (Russell and Orndorff, 1982; Jones et al., 1995). The FimH adhesin comprises a C-terminal pilin domain involved in the incorporation of FimH into the tip fibrillum and an N-terminal adhesin domain (Fig. 1A) (Choudhury et al., 1999). A carbohydrate-binding pocket localized at the distal tip of the adhesin domain can mediate bacte-
affinity for monomannose residues often involves mutations within the bottom part of the adhesin domain that are likely to alter the conformation and stability of the protein loops that carry the receptor-interacting residues (Schembri et al., 2000). The monomannose-binding phenotype prevalent among UPEC isolates confers a higher tropism for glycoprotein receptors expressed by uroepithelial cells and enhances bacterial colonization of the urinary tract (Sokurenko et al., 1998). FimH-mediated bacterial interactions with uroepithelial cells are critical to the ability of UPEC to colonize the bladder and cause disease (Connell et al., 1996; Thankavel et al., 1997; Langermann et al., 1997; Mulvey et al., 1998). The primary host receptor for type 1 pili within the bladder appears to be an integral membrane glycoprotein known as uroplakin 1a (UP1a) (Zhou et al., 2001). This protein, together with three other partners (UP1b, UPII and UPIII), are assembled into 16 nm diameter hexagonal complexes that are further organized into plaques 0.3–0.5 μm in diameter (Sun et al., 1996). These plaques cover almost the entire lumenal surface of the bladder and are thought to function as part of a permeability barrier and may help strengthen and stabilize the bladder epithelial cells. Expression of the uroplakin plaques by the superficial epithelial cells that line the bladder lumen make these cells a primary target for UPEC upon entering the urinary tract (Mulvey et al., 1998). Bacterial interactions with a sparse glycolcalyx that overifies the bladder surface may also facilitate bacterial colonization of the urinary tract (Hopkins et al., 1990).

While monomannose binding by FimH enhances bacterial interactions with uroplakin receptors and facilitates colonization of the lower urinary tract, it may also impair the fitness of UPEC at other sites within the host. For example, type 1 pili appear to assist bacterial transmission between hosts by promoting transient colonization of the oropharyngeal cavity, the initial portal of entry for UPEC and other E. coli isolates (Bloch et al., 1992). Here, bacteria encounter a number of soluble inhibitors that interact more effectively with monomannose-binding FimH variants relative to FimH forms that bind only trimannose residues (Sokurenko et al., 1998). Therefore, at this location, expression of FimH variants having monomannose-binding affinity may be a disadvantage to the survival and transmission of UPEC. Even within the urinary tract, the expression of monomannose-binding FimH variants by UPEC can potentially complicate bacterial colonization by increasing the affinity of FimH for soluble glycoprotein receptors within the urine. One of the most abundant urinary proteins, the Tamm–Horsfall protein, has recently been shown to preferentially bind E. coli strains expressing monomannose-binding variants of FimH and so can prevent type 1 pili-mediated bacterial adherence to uroepithelial cells (Pak et al., 2001).

In addition to promoting bacterial–host interactions, recent studies have demonstrated that some FimH variants can also mediate interbacterial contacts, stimulating bacterial autoaggregation and biofilm formation (Pratt and Kolter, 1998; Schembri and Klemm, 2001a; Schembri et al., 2001). The role of FimH in these processes is not yet clear, but they do not seem to necessarily depend on the mannose-binding capacity of the adhesin. FimH-mediated autoaggregation and biofilm formation may enable UPEC to better withstand antibiotic treatments and host antibacterial defences within the urinary tract. In addition, type 1 pilus-mediated biofilm formation may facilitate bacterial colonization of urinary catheters and other medical...
implants, an unfortunately common problem for hospitalized individuals.

P pili

Similar to type 1 pili, P pili are also composite organelles consisting of a short, flexible tip fibrillum attached to the distal end of a thicker rod structure (Kuehn et al., 1992; Bullitt and Makowski, 1995). The P pilus rod is 6.8 nm wide and is composed of repeating PapA subunits arranged in a right-handed helical cylinder. A subunit designated PapH is thought to anchor the PapA rod to the outer membrane (Baga et al., 1987). The tip fibrillum, which is about 2 nm thick, contains a distally located adhesin, PapG, in association with three other subunits, PapE, PapF, and PapK (Kuehn et al., 1992; Jacob-Dubuisson et al., 1993). The PapG adhesin recognizes glycolipid receptors expressed by erythrocytes and host cells present in the kidney (Leffler and Svanborg-Eden, 1993). Studies have indicated that P pili, and specifically the PapG adhesin, are significant virulence factors associated with pyelonephritis. Roberts and colleagues found that PapG, while unnecessary for UPEC colonization of the bladder, is essential for a pyelonephritic UPEC isolate to adhere to renal tissue and cause pyelonephritis in cynomogus monkeys (Roberts et al., 1994). More recently, Wullt et al. (2000) demonstrated using human volunteers that P pilus enhance the colonization of the urinary tract and facilitate the establishment of bacteriuria by an UPEC isolate known to cause asymptomatic bacteriuria. Interestingly, epidemiological studies indicate that while P pili are important factors in initiating pyelonephritis in normal urinary tracts, these adhesive organelles seem to have a less significant role in colonizing urinary tracts with abnormalities or obstructions (Jantunen et al., 2000; Tseng et al., 2001). Outside the urinary tract, a clear-cut role for P pilus expression has not been established. However, Alpers and coworkers have recently demonstrated that the PapG adhesin can bind surfactant-like particles that are secreted by human and mouse intestines (Goetz et al., 1999; Mahmood et al., 2000). PapG-mediated interactions with these particles, it is proposed, may enable UPEC to establish a reservoir within the intestinal mucosa and could facilitate UPEC persistence among the intestinal flora.

The minimal glycolipid receptor for the PapG adhesin is called globotriacylsphingosylceramide (GbO3) and consists of a digalactoside (Galα1–4Gal) core linked by a β-glucose (Glc) residue to a ceramide group that anchors the receptor in the membrane (Stromberg et al., 1990; 1991). Alteration of the Galα1–4Gal core of GbO3 by the addition of a single N-acetyl-galactosamine (GalNAc) generates GbO4 (globoside), while modification with two GalNAc sugars creates GbO5 (the Forssmann antigen). Three distinct PapG variants, designated GI, GII and GIII, have been identified that recognize GbO3, GbO4 and GbO5 respectively. It has been suggested that the different PapG variants affect the host specificity of pyelonephritic UPEC strains, but recent epidemiological studies have raised doubts regarding this possibility (Feria et al., 2001).

Recently, both the crystal and solution structures of the PapGII adhesin domain were reported (Dodson et al., 2001; Sung et al., 2001). This domain, which shares little sequence homology with the adhesin domain of FimH, is a mostly β-sheet structure consisting of an open and elongated β-sandwich having long loops and a short helical section connecting one set of the loops (Fig. 1B). Co-crystallization of PapGII with GbO4, along with mutation studies, revealed that the GbO4 receptor binds along the side of PapG via a rigid body type interaction that does not involve conformational alterations in the adhesin. Side-on binding of PapG with its receptor may promote optimal interactions with oligosaccharide receptors that are proposed to be kinked about the lipid moiety of GbO4 (Gronberg et al., 1994; Sung et al., 2001). In addition, the tips of P pili appear to be fairly flexible and this flexibility may enhance side-on interactions between PapG and glycolipid receptors embedded within host cell membranes (Kuehn et al., 1992).

S/F1C pili

S pili have a similar, although less well-defined, architecture to type 1 and P pili (Schmoll et al., 1989). S pilus fibers are composed of a major subunit SfaA in addition to three minor subunits SfaG, SfaH and SfaS. The SfaS subunit has been localized to S pilus tips and can mediate bacterial interactions with sialic acid residues on receptors expressed by kidney epithelial and vascular endothelial cells (Korhonen et al., 1986; Moch et al., 1987; Morschhauser et al., 1990; Hanisch et al., 1993). The major subunit SfaA also has adhesin characteristics and can mediate bacterial adherence to endothelial cell glycolipids and plasminogen (Parkkinnen et al., 1991; Prasadaraao et al., 1993). Minor subunits, in addition to SfaS, may also modulate the binding properties of S pili (Schmoll et al., 1989; Morschhauser et al., 1993). S pili may facilitate bacterial dissemination within host tissues and are often associated with E. coli strains that cause sepsis, meningitis, and ascending UTIs, including pyelonephritis (Korhonen et al., 1985; Marre et al., 1986; Parkkinnen et al., 1988; Hacker et al., 1993). Recent work showing that sialic acid residues are presented on UP3, one of four integral membrane uroplakin proteins.

© 2002 Blackwell Science Ltd, Cellular Microbiology, 4, 257–271
abundantly expressed on the luminal surface of the bladder, suggests that S pili may also have a role in cystitis (Malagolini et al., 2000). A number of adhesive organelles have been identified which are genetically homologous to S pili, but differ in their receptor specificity (Ott et al., 1988; Hacker et al., 1993). Among these homologous structures are F1C pili which can bind β-GalNac-1, 4β-Gal residues on glycolipids expressed by epithelial cells of the distal tubules and collecting ducts of the kidney and also by bladder and kidney endothelial cells (Khan et al., 2000). F1C pili are encoded by approximately 14% of UPEC isolates. This observation, along with the binding specificity of these organelles, indicates that F1C pili may impact the pathogenesis of a significant number of UTI cases.

Dr adhesin family

This group of adhesins includes the uropathogen-associated fimbrial adhesin Dr and the non-fimbrial adhesins AFA-I, AFA-II, AFA-III, AFA-IV, Nfa-I and Dr-II (reviewed in Nowicki et al., 2001). Dr adhesin family members are proposed to facilitate ascending colonization and chronic interstitial infection of the urinary tract. Epidemiological studies indicate that about half of all children with UTIs, and over 30% of pregnant women with pyelonephritis, are colonized by UPEC strains expressing the Dr adhesion. In addition, infection with E. coli strains expressing Dr results in a twofold increase in the risk for a recurrent UTI. The contribution of Dr as a virulence factor able to promote bacterial persistence within the urinary tract can be substantial. Recently, it was reported that UPEC encoding the Dr adhesin, but not Dr+ bacteria, could survive for more than 1 year within renal tissue (Goluszko et al., 1997b; Nowicki et al., 2001). Members of the Dr adhesion family recognize one or more of four 60-amino-acid short consensus repeat (SCR) sequences present in decay accelerating factor (DAF, CD55), a glycosylphosphatidylinositol (GPI)-linked complement receptor and regulatory factor, expressed on erythrocytes and other tissues including the uroepithelium (Nowicki et al., 2001). Different members of the Dr adhesion family appear to recognize distinct SCR sequences within DAF. Increases in DAF expression within the urinary tract during pregnancy may be responsible for the increased susceptibility of pregnant women to infection with Dr+ UPEC (Goluszko et al., 2001). Dr adhesion family members can also bind carcinoembryonic antigen (CD66e), a GPI-anchored protein with unknown functions (Guignot et al., 2000). In addition, the Dr adhesion, but not other members of this family, can recognize type IV collagen (Nowicki et al., 2001). By interacting with DAF and possibly other receptors such as type IV collagen and CD66e, Dr can promote bacterial adherence to the interstitial compartments of the kidney and so may facilitate long-term bacterial persistence within the upper urinary tract.

Regulation of UPEC adherence

The biogenesis of bacterial adhesive organelles, in general, is a tightly controlled process. The costs in energy and other resources required for expression of an adhesin must be balanced with any potential benefits that a particular adhesive organelle might provide a bacterium. Variable expression of different adhesive organelles may allow UPEC to alter its binding characteristics in response to environmental changes encountered within a host during the course of an infection. Potentially, this can greatly expand the number of host receptors with which UPEC can interact during a UTI and may facilitate bacterial dissemination within the urinary tract. Furthermore, modulation of adhesive organelles may enable UPEC to escape rapid detection by the host immune system.

Environmental cues, including changes in temperature, osmolarity, pH, oxygen tension, carbon source, and nutrient availability can alter the expression of bacterial adhesins as can the presence of iron, aliphatic amino acids, and electron acceptors other than oxygen (Low et al., 1996; van der Woude et al., 1996; Henderson et al., 1999; Blomfield, 2001; Schwan et al., 2002). These signals can affect adhesin biogenesis through global regulator proteins that can modify the transcription of adhesin and pilus genes. A number of global regulators have been identified and include integration host factor (IHF) and H-NS, a DNA-binding histone-like protein that often mediates temperature regulation of pilus synthesis. Regulation by carbon source can occur through the catabolite activator protein (CAP), while the leucine-responsive regulatory protein (Lrp) can modulate pilus expression in response to aliphatic amino acids. In addition to these and other global regulators, specific regulator proteins encoded by genes within pilus gene clusters also modulate pilus biogenesis. Multiple regulatory factors can act upon the same promoter region, switching expression of pilus and adhesin genes from on to off and vice versa. This on and off switching, known as phase variation, is often controlled by either the methylation status of a promoter region (e.g. P pilii) or by the inversion of sequence elements within a promoter region (e.g. type 1 pilii).

The regulation of adhesin biogenesis is further complicated by intersystem regulatory cross-talk between different bacterial adhesins, virulence factors and signal transduction pathways. For example, Uhlin and colleagues found that PapB, a regulator of P pilus phase variation, and its functional homologue, SfaB, from the S pilus system are able to modulate type 1 pilus expression.
forcing an increase in the on-to-off phase transition frequency of type 1 pili (Xia et al., 2000; Holden et al., 2001). Thus, promotion of the expression of P pili by PapB results in the simultaneous repression of type 1 pili. This may allow UPEC strains to conserve energy and resources by producing only the pili they need. Furthermore, exclusionary expression of one pilus type over another may limit complications arising from the simultaneous expression of homologous subunits, chaperones and ushers and may also alleviate problems with immune recognition. In other work, Schembri and Klemm demonstrated that pilus subunits can downregulate the expression of antigen 43 (Ag43), a surface protein involved in bacterial microcolony and biofilm formation (Schembri and Klemm, 2001b). The expression of type 1 and other pilus subunits, which typically contain disulfides, is proposed to indirectly promote the reduction of the global regulator OxyR, which in turn can actively repress Ag43 expression. Modulation of the thiol-disulphide status of OxyR by disulfide-containing pilus subunits may also influence other genes, including several involved in the oxidative stress response and iron uptake.

Bacterial attachment alone can also serve to modulate expression of E. coli adhesins and virulence factors. Otto et al. (2001) recently showed that type 1 pilus-mediated bacterial adherence to abiotic surfaces can stimulate the downregulation of a number of outer membrane proteins. This attachment-induced alteration of the bacterial envelope could potentially influence both the adherence and antigenic properties of the bacteria. Others have proposed that pilus-mediated adherence can also lead to a build-up of pilus subunits within the periplasm of E. coli and this could result in activation of stress response pathways, including the Cpx two-component regulatory system (Hung and Hultgren, 1998; Hung et al., 2001). This system responds to misfolded proteins within the periplasm and outer and inner membranes of E. coli and activates the expression of protein folding and degrading factors. Recent work by Hung and co-workers indicates that activation of the Cpx system can also promote P pilus expression and might modulate the synthesis of other adhesins and virulence factors (Hung et al., 2001). Factors such as OxyR and the Cpx system, along with intersystem cross-talk via specific pilus regulator proteins such as PapB, provides a means of co-ordinating pilus biogenesis with the expression of other virulence and survival genes and is likely to enhance the ability of UPEC to successfully colonize the urinary tract.

Invasion by UPEC

UPEC has not traditionally been considered an intracellular pathogen. However, research dating back to the late 1970s and a number of more recent reports indicate that UPEC can act as an opportunistic intracellular pathogen. Standard gentamicin protection assays and microscopic studies of infected rat and mouse bladders, along with studies using host cells grown in culture, have shown that bladder epithelial cells can internalize UPEC both in vivo and in vitro (Fukushi et al., 1979; McTaggart et al., 1990; Mulvey et al., 1998; Martinez et al., 2000). Similarly, investigations using cultured renal epithelial cells have demonstrated that these host cells can also internalize E. coli (Warren et al., 1988; Donnenberg et al., 1994; Palmer et al., 1997; Springall et al., 2001). The entry of UPEC into host uroepithelial cells, rather than being a deleterious event for UPEC, can provide the bacteria with a survival advantage (Mulvey et al., 1998; Mulvey et al., 2001; Springall et al., 2001). UPEC invasion of host cells within the urinary tract possibly enhances bacterial survival by providing protection from host immune defences and allowing the pathogens greater access to deeper tissue.

Early work indicated that uroepithelial cells were innately phagocytic and that bacterial adhesins, by promoting intimate contact with these host cells, facilitated bacterial uptake. More recently, some of the adhesive organelles elaborated by UPEC, along with a few host factors, have been shown to directly trigger and/or modulate bacterial entry into host cells. Specifically, both type 1 pili and Dr family adhesins have been implicated as factors that can effectively promote bacterial invasion of host cells by activating distinct host cell signalling events (Fig. 2A–C).

Type 1 pilus-mediated invasion

Type 1 pili have been shown to mediate bacterial invasion of bladder epithelial cells via a process that is completely dependent upon the FimH adhesin (Martinez et al., 2000). Internalization assays using latex beads coated with purified FimH have demonstrated that FimH is both necessary and sufficient to mediate entry into bladder epithelial cells. Beads coated with control proteins or bacteria expressing type 1 pili lacking the FimH adhesin, although able to occasionally adhere non-specifically to bladder epithelial cells, are rarely internalized. Bacterial invasion mediated by FimH appears to occur via a zipper-like mechanism in which the host cell membrane envelopes the adherent pathogen in response to direct, sequential interactions between FimH and, as yet unidentified, host receptors. Entry likely involves the induced clustering of host receptors, as suggested by transmission electron microscopy studies that show the accumulation of electron-dense material (proteins) within the host plasma membrane below FimH-coated beads and adherent bacteria as they are internalized (see fig. 3 in Martinez et al., 2000). FimH-mediated invasion is dependent upon
activation of protein tyrosine kinases, phosphoinositide-3-kinase (PI-3 kinase), and the Rho-family GTPase member Cdc42 (Fig. 2A) (Martinez et al., 2000; Martinez and Hultgren, 2002). These events correlate with the induced phosphorylation of focal adhesion kinase (FAK) and the formation of transient complexes between FAK and PI-3 kinase. The activation of another Rho-family GTPase member, Rac1, appears to occur downstream of these events and results in the formation of complexes between the cytoskeletal components α-actinin and vinculin. This signal transduction cascade may contribute to the modulation and stabilization of localized actin cytoskeletal rearrangements that are required for the envelopment and internalization of E. coli subsequent to FimH-mediated attachment.

After internalization, type 1-piliated E. coli are usually found within membrane-bound vacuoles that do not appear to merge, at least not immediately, with the lysosomal degradation pathway (Martinez et al., 2000 and unpublished observations). It is feasible that FimH interactions with host receptors allow type 1-piliated E. coli to hitchhike along a receptor/membrane-recycling pathway that does not intersect and fuse with lysosomal compartments. Once internalized, UPEC can replicate to high levels, essentially converting host bladder epithelial cells into bacterial factories (Mulvey et al., 2001). In contrast, type1-piliated E. coli lab strains, although able to invade and persist for some time within bladder epithelial cells, are unable to multiply intracellularly. These observations suggest that intracellular growth is not a general property of all E. coli strains and requires the expression of virulence factors unique to UPEC. In vivo, massive intracellular replication of UPEC is limited to the highly differentiated superficial cells that line the lumenal surface of the bladder mucosa, even though the bacteria have the capacity to invade the less differentiated underlying epithelial cells. Such observations suggest that signals originating from the infected host cells might also modulate the ability of UPEC to grow intracellularly.

In addition to mediating bacterial invasion of bladder epithelial cells, the FimH adhesin has also been shown to promote bacterial uptake by neutrophils, mast cells and macrophages (Fig. 2B). Internalization by mast cells and macrophages is initiated as a consequence of interactions
between FimH and the GPI-anchored receptor CD48 (Baorto et al., 1997; Shin et al., 2000). The internalization process is dependent upon actin cytoskeletal alterations and protein kinase C, an enzyme known to modulate a number of endocytic processes (Lin et al., 1999). Recent work has suggested that FimH-mediated internalization by mast cells, and possibly macrophages, also involves subcellular structures known as caveolae (Baorto et al., 1997; Shin et al., 2000). These fluid structures can be considered a class of membrane domains known as lipid rafts that are enriched in both cholesterol and GPI-anchored proteins (Mulvey and Hultgren, 2000; Couet et al., 2001). Caveolae, by most definitions, are also enriched in integral membrane proteins called caveolins. The exact function of caveolin is unclear, but they are proposed to function in the import and transcytosis of macromolecules and in the formation and stabilization of signal transduction complexes. Abraham and coworkers found that caveolin and cholesterol are actively recruited to sites of bacterial entry into mast cells (Shin et al., 2000). In addition, depletion of cholesterol and the subsequent disruption of lipid rafts or caveolae-like domains using drugs such as filipin and methyl-β-cyclodextrin was found to effectively inhibit FimH-mediated bacterial uptake by both mast cells and macrophages. Based on such observations, it has been proposed that FimH-mediated bacterial internalization by these immune cells entails remodelling of plasmalemmal caveolea-like domains and the recruitment of vesicular caveolea-like particles to form membrane-bound vacuoles that encapsulate E. coli. This FimH-dependent internalization process, by trafficking E. coli away from the classic endosome-lysosome pathway, used by phagocytes to take in opsonized microbes, could conceivably enhance intracellular bacterial survival. However, this possibility is countered by data that indicate that type 1-piliated E. coli are killed at a similar rate as opsonized E. coli following internalization by macrophages (Hamrick et al., 2000b).

Entry mediated by Dr adhesins

Members of the Dr family of adhesins have been shown to promote E. coli entry into a number of epithelial-like cells, including Hela, CHO, and Caco-2 intestinal cells (Goluszko et al., 1997a; Jouve et al., 1997; Selvarangan et al., 2000; Goluszko et al., 2001; Guignot et al., 2001; Nowicki et al., 2001). Entry occurs via a zipper-like mechanism and, in most cases, is dependent upon the SCR3 region and GPI anchor of DAF (Fig. 2C). Both DAF and CD66e, an additional receptor for Dr-like adhesins, have been observed to cluster around adherent E. coli (Goluszko et al., 1999; Guignot et al., 2000; Selvarangan et al., 2000). The GPI anchors of these receptors may facilitate clustering by enhancing the lateral mobility of DAF and CD66e within the host plasma membrane. Receptor clustering, in turn, may affect the assembly and activation of host signalling molecules and complexes that could modulate cytoskeletal alterations that lead to bacterial uptake. The internalization event involves the redistribution of the cytoskeleton-associated factors ezrin and α-actinin along with rearrangements of both microtubules and F-actin. Interactions with DAF, perhaps within caveolea-like domains, could potentially modulate the entry process by stimulating Ca²⁺-dependent signalling and activating protein tyrosine kinases, PIP-3 kinase, protein kinase C, and phospholipase Cγ (Peiffer et al., 1998; Guignot et al., 2001). It has been noted that the Dr adhesin family member Afa-III is also able to mediate bacterial entry via a DAF- and CD66e-independent pathway that may involve αvβ3 integrin (Guignot et al., 2001). Bacteria that enter epithelial-like cells as a consequence of host receptor interactions with Dr-like adhesins are found within tight membrane-bound vacuoles and do not appear to be killed immediately (Goluszko et al., 1997a; Jouve et al., 1997; Selvarangan et al., 2000). It is possible that bacterial invasion of uroepithelial cells mediated by Dr and related adhesins contributes to the ability of some UPEC isolates to colonize and persist long-term within the upper urinary tract.

Ulterior entry mechanisms

Whereas the ability of type 1 pili and Dr-related adhesins to mediate host cell invasion provides direct means for UPEC to invade host cells within the urinary tract, UPEC may also use additional invasive strategies. S pili are thought to facilitate bacterial suffusion across cellular barriers and this may contribute to bacterial dissemination during sepsis and neonatal meningitis (Parkkinen et al., 1991; Prasadara et al., 1993). Whether S pili directly stimulate bacterial invasion of uroepithelial cells is not clear. Likewise, the ability of P pili to specifically promote bacterial invasion of host cells has also not been extensively studied (Martinez et al., 2000). However, the expression of P or S pili in conjunction with toxins such as α-haemolysin and cnf1 has been proposed to facilitate bacterial dissemination within host tissues (Goluszko et al., 1997a; 2001). Destruction of host cells by toxins expressed by UPEC could conceivably expose deeper tissues within the urinary tract to which P or S pili, and potentially other adhesins could then adhere. The presence or absence of capsule and the other surface components may also influence the ability of UPEC to enter host cells.

In addition to pathogen-encoded adhesins and toxins, factors secreted by the host may also positively influence the ability of UPEC to invade cells and tissues within the urinary tract. Springall et al. (2001) recently demonstrated
that opsonization of *E. coli* with the complement factor C3 enhances bacterial interactions with the proximal tubular epithelium of the kidney and augments internalization of bacteria by proximal tubular epithelial cells (PTEC). These cells are the primary targets of UPEC during the acute phase of pyelonephritis (Uhlen et al., 2000). PTEC are a major source of secreted C3, which is induced up to 400-fold after infection with UPEC or LPS administration (Springall et al., 2001). Interactions between C3 and the complement receptor CR1-related protein y (Cry) appear to mediate bacterial internalization by PTEC (Fig. 2D). Data showing that C3-deficient mice are better protected from ascending renal infection relative to wild-type mice suggest that C3-mediated bacterial internalization enhances UPEC colonization and invasion of the kidney. These results are surprising considering that C3 opsonization is known to promote bacterial phagocytosis and killing by macrophages and neutrophils. C3-mediated bacterial entry into PTEC thus highlights one way in which UPEC is able to turn the tables on an innate host defence in order to gain access to deeper tissue within the urinary tract.

**Consequences of UPEC adherence and invasion**

The presence of bacteria or bacterial products within the urinary tract can trigger rapid and robust responses from the host. Prominent among these responses during the first several hours after infection is the production of cytokines and the influx of neutrophils, which in mouse UTI models have been observed to specifically target infected uroepithelial cells (Mulvey et al., 2000; Schilling et al., 2001). Interactions between C3 and the host epithelial and immune cells stimulate the expression of a number of pro-inflammatory molecules, including interleukin 6 (IL-6), IL-8, IL-8 receptor, tumour necrosis factor α and inducible nitric oxide synthase (Steadman et al., 1988; Svanborg et al., 1996; Funfstuck et al., 1997; Poljakovic et al., 2001; Schilling et al., 2001; Mysorekar et al., 2002). IL-8 and the IL-8 receptor are key components in transepithelial migration of neutrophils during a UTI (Godaly et al., 1997; 1998; 2001; Hang et al., 1999; Frendeus et al., 2001a). Pattern recognition molecules, which include the toll-like receptors (TLRs), enable the epithelial cells lining the urinary tract to detect and respond to conserved bacterial features such as LPS and lipoprotein (Haraoka et al., 1999; Schilling et al., 2001; Svanborg et al., 2001). TLR4, which recognizes LPS, is required to mount an effective inflammatory response after infection with UPEC. In mice expressing non-functional TLR4, neutrophils are not recruited to sites of infection and bacteria are not effectively cleared from the urinary tract.

Tissues within the urinary tract appear to respond differentially to inflammatory stimuli (Backhed et al., 2001). This is, in part, due to variations in the expression of pattern recognition molecules by different host cell populations. For example, bladder epithelial cells express TLR4 and respond avidly to LPS whereas renal epithelial cells, which express little TLR4, are hyporesponsive. Adhesive organelles, especially type 1 and P pili, can greatly boost cytokine responses by facilitating the delivery of bacterial products such as LPS to host cells (Godaly et al., 1998; Hedlund et al., 2001; Schilling et al., 2001; Mysorekar et al., 2002). Bacterial invasion mediated by pili adhesins can also augment LPS-dependent uroepithelial cytokine responses, perhaps by inducing TLR clustering and internalization, or by activating pathways that synergize with signals generated by LPS (Schilling et al., 2001). Once internalized, UPEC could activate cytokine responses by stimulating intracellular TLR-independent pathways, such as the LPS-dependent CARD4/NOD1 pathway that is triggered by invasive *Shigella flexneri* (Girardin et al., 2001). Pili components may also be able to directly engage TLRs and initiate inflammatory responses independent of LPS or other bacterial factors (Hedlund et al., 1999; Frendeus et al., 2001b; Svanborg et al., 2001). However, direct stimulation of cytokine responses by adhesins expressed by UPEC in the absence of any other bacterial factors has not yet been conclusively demonstrated. The ability of pili-mediated bacterial attachment to stimulate uroepithelial cells, beyond that of LPS alone, means that host cells within the urinary tract, and especially LPS hyporesponsive cells like those found within the renal cortex, are triggered to initiate maximal inflammatory responses only when bacteria are directly encountered (Backhed et al., 2001). This may minimize collateral damage to host tissues due to pro-inflammatory processes by restricting robust inflammation to sites that are immediately threatened by adherent UPEC.

Within the bladder, the induced exfoliation of infected epithelial cells represents another hallmark host response to interactions with UPEC. The bladder epithelium normally has an exceptionally slow turnover rate of approximately 40 weeks in both mice and humans (Jost, 1989). However, large numbers of exfoliated bladder epithelial cells can often be found within urine from human patients with UTIs and from rodents with experimentally induced cystitis (Fukushi et al., 1979; Elliott et al., 1985; McTaggart and Elliott, 1989; McTaggart et al., 1990). In a mouse UTI model, massive exfoliation of bladder epithelial cells is induced within six hours after transurethral inoculation with *E. coli* expressing functional type 1 pili (Fig. 3) (Mulvey et al., 1998). Massive exfoliation in this system depends on FimH-mediated bacterial interactions with the bladder surface, but does not require either viable bacteria or functional TLR4 (Mulvey et al., 1998 and unpublished observations). This FimH-dependent exfoliation...
process occurs via an apoptosis-like pathway involving the activation of proteolytic enzymes known as caspases and host cell DNA fragmentation (Mulvey et al., 1998; Klumpp et al., 2001).

Exfoliation, similar to C3 opsonization of UPEC in the upper urinary tract (as described above), seems to represent a double-edged sword for both the host and the invading pathogens. The release of infected bladder epithelial cells and their eventual clearance from the host with the flow of urine serves as a fairly effective antibacterial defence (Mulvey et al., 1998; 2000). Treatment of mice with a pan-caspase inhibitor slows down exfoliation in response to infection and concomitantly inhibits bacterial clearance from the bladder. The release of infected bladder epithelial cells exposes underlying epithelial cells, making them more susceptible to infection. Indeed, a recent study by Klumpp et al. (2001) suggests that UPEC actively promotes host cell death and exfoliation by down-regulating both the NF-κB and MAP kinase pathways in bladder epithelial cells. Bacteria that manage to escape from dying superficial cells before the exfoliation process is completed can go on to infect surrounding and underlying tissue (Fig. 2A) (Mulvey et al., 2001). This may not only promote bacterial dissemination within the urinary tract, but could also allow UPEC to enter a sheltered environment within the bladder where bacteria can persist for long intervals. Mouse studies support this possibility, indicating that UPEC can survive at low levels within bladder tissue in a seemingly quiescent state for weeks to months undetected by immunosurveillance mechanisms and often protected from antibiotic treatments (Hvidberg et al., 2000; Mulvey et al., 2001). Such low-level infections in humans may go unnoticed by standard clinical assays and could be a significant source for recurrent acute UTIs that plague many women throughout their lives.

Conclusion

Recently, Manges et al. (2001) documented the widespread distribution in the United States of a clonal group of UPEC strains with resistance to trimethoprim-sulphamethoxazole, an antibiotic combination commonly used to treat UTIs. The authors suggest that this clonal group may have originated from a common source such as contaminated food. This and similar observations from other countries suggest that we have a long way to go in deciphering the transmission and virulence mechanisms of UPEC. More extensive epidemiological and clinical studies, along with advanced cell culture and animal models, structural biology, microbial genetics and emerging technologies such as DNA microarrays are beginning to provide exceptional insight into the mechanisms and consequences of UPEC interactions with its host. This work, it is hoped, will eventually lead to the development of more efficacious antibacterial treatments not only for UTIs, but also for other bacterial infections.

© 2002 Blackwell Science Ltd, Cellular Microbiology, 4, 257–271
Acknowledgements

I am grateful to C. Veltri, M. Veith, and H. Schubert for their expert assistance with the images. I would also like to thank S. J. Hultgren for all of his help. Research in my laboratory is supported by NIH grant AI01807-01.

References

Backhed, F., Soderhall, M., Ekman, P., Normark, S., and Richter-Dahlfors, A. (2001) Induction of innate immune responses by Escherichia coli and purified lipopolysaccharide correlate with organ- and cell-specific expression of Toll-like receptor 4 in the human urinary tract. Cell Microbiol 3: 153–158.

Baga, M., Norgren, M., and Normark, S. (1987) Biogenesis of E. coli Pap pili: PapH, a minor pilin subunit involved in cell anchoring and length modulation. Cell 49: 241–251.

Baorto, D.M., Gao, Z., Malaviya, R., Dustin, M.L., van der Merwe, A., Lublin, D.M., and Abraham, S.N. (1997) Survival of FimH-expressing enterobacteria in macrophages relies on glycolipid traffic. Nature 389: 636–639.

Bloch, C.A., Stocker, B.A., and Omdorf, P.E. (1992) A key role for type 1 pili in enterobacterial communicability. Mol Microbiol 6: 697–701.

Blomfield, I.C. (2001) The regulation of pap and type 1 fimbriation in Escherichia coli. Adv Microb Physiol 45: 1–49.

Brinton, C.C. (1959) Non-flagellar appendages of bacteria. Nature 183: 782–786.

Buchanan, K., Falkow, S., Hull, R.A., and Hull, S.I. (1985) Frequency among Enterobacteriaceae of the DNA sequences encoding type 1 pili. J Bacteriol 162: 799–803.

Bullitt, E., and Makowski, L. (1995) Structural polymorphism of bacterial adhesion pili. Nature 373: 164–167.

Choudhury, D., Thompson, A., Stojanoff, V., Langermann, S., Pinkner, J., Hultgren, S.J., and Knight, S.D. (1999) X-ray structure of the FimC-FimH chaperone-adenosine complex from uropathogenic Escherichia coli [see comments]. Science 285: 1061–1066.

Connell, H., Agace, W., Klemm, P., Schembri, M., Marild, S., and Svanborg, C. (1996) Type 1 fimbrial expression enhances Escherichia coli virulence for the urinary tract. Proc Natl Acad Sci USA 93: 9827–9832.

Couet, J., Belanger, M.M., Roussel, E., and Drolet, M.C. (2001) Cell biology of cavelae and caveolin. Adv Drug Deliv Rev 49: 223–235.

Dodson, K.W., Pinkner, J.S., Rose, T., Magnusson, G., Hultgren, S.J., and Waksman, G. (2001) Structural basis of the interaction of the pyelonephritic E. coli adhesin to its human kidney receptor. Cell 105: 733–743.

Donnenberg, M.S., Newman, B., Utsalo, S.J., Trillifs, A.L., Hebel, J.R., and Warren, J.W. (1994) Internalization of Escherichia coli into human kidney epithelial cells: comparison of fecal and pyelonephritis-associated strains. J Infect Dis 169: 831–838.

Elliott, T.S., Reed, L., Slack, R.C., and Bishop, M.C. (1985) Bacteriology and ultrastructure of the bladder in patients with urinary tract infections. J Infect 11: 191–199.

Feria, C., Machado, J., Duarte Correia, J., Goncalves, J., and Gaastra, W. (2001) Distribution of papaG alleles among uropathogenic Escherichia coli isolated from different species. FEMS Microbiol Lett 202: 205–208.

Foxman, B., Zhang, L., Talman, P., Palin, K., Rode, C., Bloch, C., et al. (1995) Virulence characteristics of Escherichia coli causing first urinary tract infection predict risk of second infection. J Infect Dis 172: 1536–1541.

© 2002 Blackwell Science Ltd, Cellular Microbiology, 4, 257–271
phatidylinositol-anchored proteins by members of the Afa/Dr diffusely adhering family of *Escherichia coli* that infect the human polarized intestinal Caco-2/TC7 cells. * Infect Immun* **68**: 3554–3563.

Guignot, J., Bernet-Camard, M.F., Pous, C., Plancon, L., Le Bouguenec, C., and Servin, A.L. (2001) Polarized entry of uropathogenic Afa/Dr diffusely adhering *Escherichia coli* strain IH11128 into human epithelial cells: evidence for alpha5beta1 integrin recognition and subsequent internalization through a pathway involving caveloae and dynamic unstable microtubules. * Infect Immun* **69**: 1856–1868.

Hacker, J., Kestler, H., Hoschutzky, H., Jann, K., Lottspeich, F., and Korhonen, T.K. (1993) Cloning and characterization of the S fibmral adhesin IH1 complex of an *Escherichia coli* O18: K1 meningitis isolate. * Infect Immun* **61**: 544–550.

Hacker, J., and Kaper, J.B. (2000) Pathogenicity islands and the evolution of microbes. * Annu Rev Microbiol* **54**: 641–679.

Hamrick, T.S., Harris, S.L., Spears, P.A., Havell, E.A., Horton, J.R., Russell, P.W., and Orndorff, P.E. (2000a) Genetic characterization of *Escherichia coli* type 1 pilus adhesion mutants and identification of a novel binding phenotype. * J Bacteriol* **182**: 4012–4021.

Hamrick, T.S., Havell, E.A., Horton, J.R., and Orndorff, P.E. (2000b) Host and bacterial factors involved in the innate ability of mouse macrophages to eliminate internalized unopsonized *Escherichia coli*. * Infect Immun* **68**: 125–132.

Hang, L., Haraoka, M., Agace, W.W., Leffler, H., Burdick, M., Strieter, R., and Svannborg, C. (1999) Macrophage inflammatory protein-2 is required for neutrophil passage across the epithelial barrier of the infected urinary tract. * J Immunol** **162**: 3037–3044.

Hanisch, F.G., Hacker, J., and Schroten, H. (1993) Specificity of S fimbrin on recombinant *Escherichia coli*: preferential binding to gangliosides expressing NeuGc alpha (2–3) Gal and NeuAc alpha (2–8) NeuAc. * Infect Immun* **61**: 2108–2115.

Haraoka, M., Hang, L., Frendus, B., Godaly, G., Burdick, M., Strieter, R., and Svannborg, C. (1999) Neutrophil Recruitment and Resistance to Urinary Tract Infection. * J Infect Dis** **180**: 1220–1229.

Harris, S.L., Spears, P.A., Havell, E.A., Hamrick, T.S., Horton, J.R., and Orndorff, P.E. (2001) Characterization of *Escherichia coli* type 1 pilus mutants with altered binding specificities. * J Bacteriol** **183**: 4099–4102.

Hedlund, M., Wachtler, C., Johansson, E., Hang, L., Somerville, J.E., Darveau, R.P., and Svannborg, C. (1999) P fimbrin-dependent, lipopolysaccharide-independent activation of epithelial cytokine responses [Process Citation]. * Mol Microbiol** **33**: 693–703.

Hedlund, M., Frendus, B., Wachtler, C., Hang, L., Fischer, H., and Svannborg, C. (2001) Type 1 fimbrin deliver an LPS- and TLR4-dependent activation signal to CD14-negative cells. * Mol Microbiol** **39**: 542–552.

Henderson, I.R., Owen, P., and Nataro, J.P. (1999) Molecular switches – the ON and OFF of bacterial phase variation. * Mol Microbiol** **33**: 919–932.

Holden, N.J., Uhlin, B.E., and Gally, D.L. (2001) PapB paralogues and their effect on the phase variation of type 1 fimbrin in *Escherichia coli*. * Mol Microbiol** **42**: 319–330.

Hooton, T.M., and Stamm, W.E. (1997) Diagnosis and treatment of uncomplicated urinary tract infection. * Infect Dis Clin North Am** **11**: 551–581.

Hopkins, W.J., Reznikoff, C.A., Oberley, T.D., and Uehling, D.T. (1990) Adherence of uropathogenic E. coli to differentiated human uroepithelial cells grown in vitro. * J Urol** **143**: 146–149.

Hultgren, S.J., Jones, C.H., and Normark, S.N. (1996) Bacterial Adhesins and Their Assembly. In: *Escherichia coli and Salmonella: Cellular and Molecular Biology*. Neidhardt, F.C. (ed.). Washington DC: American Society for Microbiology Press, pp. 2730–2756.

Hung, D.L., and Hultgren, S.J. (1998) Pilius biogenesis via the chaperone/usher pathway: an integration of structure and function. * J Struct Biol** **124**: 201–220.

Hung, D.L., Rainio, T.L., Jones, C.H., Silhavy, T.J., and Hultgren, S.J. (2001) Cpx signaling pathway monitors biogenesis and affects assembly and expression of P pili. * EMBO J** **20**: 1508–1518.

Hung, C.-S.W., Bouckaert, J., Hung, D.L., Pinkner, J.S., Widberg, C., DeFusco, A., and et al. (2002) Structural basis of tropism of *Escherichia coli* to the bladder during urinary tract infection. * Mol Microbiol** (in press).

Hvidberg, H., Struve, C., Kroghel, K.A., Christensen, N., Rasmussen, S.N., and Frimodt-Moller, N. (2000) Development of a long-term ascending urinary tract infection mouse model for antibiotic treatment studies. * Antimicrob Agents Chemother** **44**: 156–163.

Jacob-Dubuisson, F., Heuser, J., Dodson, K., Normark, S., and Hultgren, S. (1993) Initiation of assembly and association of the structural elements of a bacterial pilus depend on two specialized tip proteins. * EMBO J** **12**: 837–847.

Jantunen, M.E., Siltone, A., Koskimies, O., Wikström, S., A., Salo, U., and, K., E.and Saxen, H. (2000) Predominance of class II papG allele of *Escherichia coli* in pyelonephritis in infants with normal urinary tract anatomy. * J Infect Dis** **181**: 1822–1824.

Johnson, J.R. (1991) Virulence factors in *Escherichia coli* urinary tract infection. * Clin Microbiol Rev** **4**: 80–128.

Johnson, D.E., Lockattel, C.V., Russell, R.G., Hebel, J.R., Island, M.D., Stapleton, A., et al. (1998) Comparison of *Escherichia coli* strains recovered from human cystitis and pyelonephritis in transurethrally challenged mice. * Infect Immun** **66**: 3059–3065.

Jones, C.H., Pinkner, J.S., Roth, R., Heuser, J., Nichols, A.V., Abraham, S.N., and Hultgren, S.J. (1995) FimH adhesin of type 1 pill is assembled into a fibrillar tip structure in the Enterobacteriaceae. * Proc Natl Acad Sci USA** **92**: 2081–2085.

Jost, S.P. (1989) Cell cycle of normal bladder urothelium in developing and adult mice. *Vircows Arch B Cell Pathol Incl Mol Pathol** **57**: 27–36.

Jouve, M., Garcia, M.I., Courcoux, P., Labigne, A., Gounon, P., and Le Bouguenec, C. (1997) Adhesion to and invasion of HeLa cells by pathogenic *Escherichia coli* carrying the afa-3 gene cluster are mediated by the AfaE and AfaD proteins, respectively. * Infect Immun** **65**: 4082–4089.

Khan, A.S., Kniep, B., Oelschlager, T.A., Van Die, I., Korhonen, T., and Hacker, J. (2000) Receptor structure for F1C fimbrae of uropathogenic *Escherichia coli*. * Infect Immun** **68**: 3541–3547.

Klemm, P., Christiansen, G., Kreft, B., Marre, R., and Bergmans, H. (1994) Reciprocal exchange of minor components of Type 1 and F1C fimbrae results in hybrid organelles with changed receptor specificities. * J Bacteriol** **176**: 2227–2234.

Klump, D.J., Weiser, A.C., Sengupta, S., Forrestal, S.G., Batler, R.A., and Schaeffer, A.J. (2001) Uropathogenic *Escherichia coli* Potentiates Type 1 Pilus-Induced Apoptosis by Suppressing NF-kappaB. * Infect Immun** **69**: 6689–6695.

Korhonen, T.K., Vahtonen, M.V., Parkkinen, J., Vaisanen-Rhen, V., Finne, J., Orskov, F., et al. (1985) Serotypes, hemolysin production, and receptor recognition of *Escherichia coli* strains

© 2002 Blackwell Science Ltd, *Cellular Microbiology*, 4, 257–271.
associated with neonatal sepsis and meningitis. *Infect Immun* **48**: 486–491.

Korhonen, T.K., Parkkinen, J., Hacker, J., Finne, J., Pere, A., Rhen, M., and Holthofer, H. (1986) Binding of *Escherichia coli* S fimbiae to human kidney epithelium. *Infect Immun* **54**: 322–327.

Kuehn, M.J., Heuser, J., Normark, S., and Hultgren, S.J. (1992) P pilus in uropathogenic *E. coli* are composite fibres with distinct fibrillar adhesive tips. *Nature* **356**: 252–255.

Kukkonen, M., Raunio, T., Virkola, R., Lahteenmaki, K., Makela, P.H., Klemm, P., et al. (1993) Basement membrane carbohydrate as a target for bacterial adhesion: binding of type I fimbriae of *Salmonella enterica* and *Escherichia coli* to laminin. *Mol Microbiol* **7**: 229–237.

Langermann, S., Palaszynski, S., Barnhart, M., Auguste, G., Pinkner, J.S., Burlein, J., et al. (1997) Prevention of mucosal *Escherichia coli* infection by FimH-adhesin-based systemic vaccination [see comments]. *Science* **276**: 607–611.

Leffler, H., and Svanborg-Eden, C. (1980) Chemical identification of a glycosphingolipid receptor for *Escherichia coli* attaching to human urinary tract epithelial cells and agglutinating human erythrocytes. *FEMS Microbiol Lett* **8**: 127–134.

Lin, T.J., Gao, Z., Arock, M., and Abraham, S.N. (1999) Internalization of FimH+ *Escherichia coli* by the human mast cell line (HMC-1 5C6) involves protein kinase C. *J Leukoc Biol* **66**: 1031–1038.

Low, D., Braaten, B., and van der Woude, M. (1996) Fimbriae. In: *Escherichia Coli and Salmonella*. F.C. Neidhardt (ed.), Washington DC: American Society for Microbiology Press, pp. 146–157.

Malmberg, P., Funk, H., and Malmberg, A. (1987) The PapG protein is the alpha-D-galactopyranosyl-(1–4)– beta-D-galactopyranose-binding adhesin of uropathogenic *Escherichia coli*. *Proc Natl Acad Sci USA* **84**: 5898–5902.

Mahmood, A., Engle, M.J., Hultgren, S.J., Goetz, G.S., Dodson, K., and Alpers, D.H. (2000) Role of intestinal surfactant-like particles as a potential reservoir of uropathogenic *Escherichia coli*. *Biochim Biophys Acta* **1523**: 49–55.

Malagolini, N., Cavallone, D., Wu, X.R., and Serafini-Cessi, F. (2000) Terminal glycosylation of bovine uroplakin III, one of the major integral-membrane glycoproteins of mammalian bladder. *Biochim Biophys Acta* **1475**: 231–237.

Manges, A.R., Johnson, J.R., Foxman, B., O'Bryan, T.T., Fullerton, K.E., and Riley, L.W. (2001) Widespread distribution of urinary tract infections caused by a multidrug-resistant *Escherichia coli* clonal group. *N Engl J Med* **345**: 1007–1013.

Marre, R., Hacker, J., Henkel, W., and Goebel, W. (1986) Contribution of cloned virulence factors from uropathogenic *Escherichia coli* strains to nephropathogenicity in an experimental rat pyelonephritis model. *Infect Immun* **54**: 761–767.

Martinez, J.J., Mulvey, M.A., Schilling, J.D., Pinkner, J.S., and Hultgren, S.J. (2000) Type 1 pilus-mediated bacterial invasion of bladder epithelial cells. *EMBO J* **19**: 2803–2812.

Martinez, J.J., and Hultgren, S.J. (2002) Requirement of Rho-family GTPases in the invasion of Type 1-piliated uropathogenic *Escherichia coli*. *Cell Microbiol* **4**: 19–28.

McTaggart, L.A., and Elliott, T.S. (1989) What makes microbes stick? *Lancet* **1**: 324.

McTaggart, L.A., Rigby, R.C., and Elliott, T.S. (1990) The pathogenesis of urinary tract infections associated with *Escherichia coli*, *Staphylococcus saprophyticus* and *S. epidermidis*. *J Med Microbiol* **32**: 135–141.

Moch, T., Hoschutzy, H., Hacker, J., Kroncke, K.-D., and Jann, K. (1987) Isolation and characterization of the a-sialyl-b-2,3-galactosyl-specific adhesin from fimbriated *Escherichia coli*. *Proc Natl Acad Sci USA* **84**: 3462–3466.

Morschhauser, J., Hoschutzy, H., Jann, K., and Hacker, J. (1990) Functional analysis of the sialidic acid-binding adhesin SfaS of pathogenic *Escherichia coli* by site-specific mutagenesis. *Infect Immun* **58**: 2133–2138.

Morschhauser, J., Reutter, V., Korhonen, T., Uhlin, B.E., and Hacker, J. (1993) Regulation and binding properties of S fimbiae cloned from *E. coli* strains causing urinary tract infection and meningitis. *Zentralbl Bakteriol* **278**: 165–176.

Mulvey, M.A., Lopez-Boado, Y.S., Wilson, C.L., Roth, R., Parks, W.C., Heuser, J., and Hultgren, S.J. (1998) Induction and evasion of host defenses by type 1-piliated uropathogenic *Escherichia coli*. *Science* **282**: 1494–1497.

Mulvey, M.A., and Hultgren, S.J. (2000) Cell biology. Bacterial splekunlers. *Science* **289**: 732–733.

Mulvey, M.A., Schilling, J.D., Martine, J.J., and Hultgren, S.J. (2000) Bad bugs and beleaguered bladders: interplay between uropathogenic *Escherichia coli* and innate host defenses. *Proc Natl Acad Sci USA* **97**: 8829–8835.

Mulvey, M.A., Schilling, J.D., and Hultgren, S.J. (2001) Establishment of a persistent *Escherichia coli* reservoir during the acute phase of a bladder infection. *Infect Immun* **69**: 4572–4579.

Mysorekar, I.U., Mulvey, M.A., Hultgren, S.J., and Gordon, J.I. (2002) Molecular Regulation of Uruthelial Renewal and Host Defense during Infection with Uropathogenic *Escherichia coli*. *J Biol Chem* **277**: 7412–7419.

Nowicki, B., Selvarangan, R., and Nowicki, S. (2001) Family of *Escherichia coli* Dr adhesins: decay-accelerating factor receptor recognition and invasiveness. *J Infect Dis* **183** (Suppl. 1): S24–S27.

O’Hanley, P., Lark, D., Falkow, S., and Schoolnik, G. (1985) Molecular basis of *Escherichia coli* colonization of the upper urinary tract in BALB/c mice: Gal-Gal pilus immunization prevents *Escherichia coli* pyelonephritis. *J Clin Invest* **83**: 2102–2108.

Ofek, I., Mirelman, D., and Sharon, N. (1977) Adherence of *Escherichia coli* to human mucosal cells mediated by mannose receptors. *Nature* **265**: 623–625.

Ott, M., Hoschutzy, H., Jann, K., Van Die, I., and Hacker, J. (1988) Gene clusters for S fimbrial adhesin (sfa) and F1C fimbiae (loci of *Escherichia coli*: comparative aspects structure function *J Bacteriol* **170**: 3983–3990.

Otto, K., Norbeck, J., Larsson, T., Karlsson, K.A., and Hermansson, M. (2001) Adhesion of type 1-fimbriated *Escherichia coli* to abiotic surfaces leads to altered composition of outer membrane proteins. *J Bacteriol* **183**: 2445–2453.

Park, J., Yu, Z., Zhang, Z.T., Hasty, D.L., and Wu, X.R. (2001) Tamm–Horsfall protein binds to type 1 fimbriated *Escherichia coli* and prevents *E. coli* from binding to uroplakin Ia and Ib receptors. *J Biol Chem* **276**: 9924–9930.

Palmer, L.M., Reilly, T.J., Utsalo, S.J., and Donnenberg, M.S. (1997) Internalization of *Escherichia coli* by human renal epithelial cells is associated with tyrosine phosphorylation of specific host cell proteins. *Infect Immun* **65**: 2570–2575.

Parkkinen, J., Hacker, J., and Korhonen, T.K. (1991) Enhancement of tissue plasminogen activator-catalyzed plasminogen activation by *Escherichia coli* S fimbiae associated with neonatal sepsicaemia and meningitis. *Thromb Haemostasis* **65**: 483–486.
S. (1988) Binding sites in the rat brain for Escherichia coli S-fimbriae associated with neonatal meningitis. J Clin Invest 81: 860–865.

Peiffer, I., Servin, A.L., and Bernet-Camard, M.F. (1998) Piracy of decay-accelerating factor (CD55) signal transduction by the diffusely adhering strain Escherichia coli C1845 promotes cytoskeletal F-actin rearrangements in cultured human intestinal INT407 cells. Infect Immun 66: 4036–4042.

Poljakovic, M., Svensson, M.L., Svanborg, C., Johansson, K., Larsson, B., and Persson, K. (2001) Escherichia coli-induced inducible nitric oxide synthase and cyclooxygenase expression in the mouse bladder and kidney. Kidney Int 59: 893–904.

Poultu, R., Puustinen, T., Virkola, R., Hacker, J., Klemm, P., and Korhonen, T.K. (1999) Amino acid residue Ala-62 in the FimH fimbrial adhesin is critical for the adhesiveness of meningitis-associated Escherichia coli to collagens. Mol Microbiol 31: 1747–1757.

Prasadara, N.V., Wass, C.A., Hacker, J., Jann, K., and Kim, K.S. (1993) Adhesion of S-fimbriated Escherichia coli to brain glycolipids mediated by sfaA gene encoded protein of S-fimbriae. J Biol Chem 268: 10356–10363.

Pratt, L.A., and Kolter, R. (1998) Genetic analysis of Escherichia coli biofilm formation: roles of flagella, motility, chemotaxis and type 1 pili. Mol Microbiol 30: 285–293.

Roberts, J.A., Marklund, B.-I., Ilver, D., Haslam, D., Kaack, M.B., Schwan, W.R., Lee, J.L., Lenard, F.A., Matthews, B.T., and Beck, S. (1988) Binding sites in the rat brain for Escherichia coli biofilm formation: roles of flagella, motility, chemotaxis and type 1 pili. Mol Microbiol 30: 27–34.

Russell, P.W., and Omdorf, P.E. (1992) Lesions in two Escherichia coli type 1 pilus genes alter pilus number and length without affecting receptor binding. J Bacteriol 174: 5923–5935.

Sadler, I., Chiang, A., Kurihara, T., Rothblatt, J., Way, J., and Silver, D. (1989) A yeast gene important for protein assembly into the endoplasmic reticulum and the nucleus has homology to DnaJ, an Escherichia coli heat shock protein. J Cell Biol 109: 2665–2675.

Sauer, F.G., Knight Waksman, G.J., and Hultgren, S.J. (1994) The Gal a(1–4) Gal-specific tip adhesin of Escherichia coli P-fimbriae is needed for pyelonephritis to occur in the normal urinary tract. Proc Natl Acad Sci USA 91: 11889–11893.

Schaible, M.A., Sokurenko, E.V., and Klemm, P. (2000) Functional flexibility of the FimH adhesin: insights from a random mutant library. Infect Immun 68: 2638–2646.

Schaible, M.A., Christiansen, G., and Klemm, P. (2001) FimH-mediated autoaggregation of Escherichia coli. Mol Microbiol 41: 1419–1430.

Schiembi, M.A., and Klemm, P. (2001a) Biofilm formation in a hydrodynamic environment by novel fimh variants and ramifications for virulence. Infect Immun 69: 1322–1328.

Schiembi, M.A., and Klemm, P. (2001b) Coordinate gene regulation by fimbriae-induced signal transduction. EMBO J 20: 3074–3081.

Schilling, J.D., Mulvey, M.A., Vincent, C.D., Lorenz, R.G., and Hultgren, S.J. (2001) Bacterial invasion augments epithelial cytokine responses to Escherichia coli through a lipopolysaccharide-dependent mechanism. J Immunol 166: 1148–1155.

Schmoll, T., Hoschutzyk, H., Morschhauser, J., Lottspeich, F., Jann, K., and Hacker, J. (1989) Analysis of genes coding for the sialic acid-binding adhesin and two other minor fimbrial subunits of the S-fimbrial adhesin determinant of Escherichia coli. Mol Microbiol 3: 1735–1744.

Schwan, W.R., Lee, J.L., Lenard, F.A., Matthews, B.T., and Beck, M.T. (2002) Osmolarity and pH Growth Conditions Regulate fim Gene Transcription and Type 1 Pilus Expression in Uropathogenic Escherichia coli. Infect Immun 70: 1391–1402.

Selvarangan, R., Goluszko, P., Popov, V., Singhal, J., Pham, T., Lublin, D.M., et al. (2000) Role of decay-accelerating factor domains and anchorage in internalization of Dr-fimbriated Escherichia coli. Infect Immun 68: 1391–1399.

Shin, J.S., Gao, Z., and Abraham, S.N. (2000) Involvement of cellular caveolae in bacterial entry into mast cells. Science 289: 785–788.

Sobel, J.D. (1997) Pathogenesis of urinary tract infection. Role of host defenses. Infect Dis Clin North Am 11: 531–549.

Sokurenko, E.V., Sande, J.S., Ohman, D.E., Klemm, P., and Hasty, D.L. (1994) FimH family of type 1 fimbrial adhesins: functional heterogeneity due to minor sequence variations among fimH genes. J Bacteriol 176: 748–755.

Sokurenko, E.V., Courtney, H.S., Ohman, D.E., Klemm, P., and Hasty, D.L. (1995) Quantitative differences in adhesiveness of type 1 fimbriated Escherichia coli due to structural differences in fimH genes. J Bacteriol 177: 3680–3686.

Sokurenko, E.V., Chesnokova, V., Doyle, R.J., and Hasty, D.L. (1997) Diversity of the Escherichia coli type 1 fimbrial lectin. Differential binding to mannosides and ureepithelial cells. J Biol Chem 272: 17880–17886.

Sokurenko, E.V., Chesnokova, V., Dykuizen, D.E., Ofek, I., Wu, X.R., Krogfelt, K.A., et al. (1998) Pathogenic adaptation of Escherichia coli by natural variation of the FimH adhesin. Proc Natl Acad Sci USA 95: 8922–8926.

Sokurenko, E.V., Schembri, M.A., Trintchina, E., Kjaergaard, K., Hasty, D.L., and Klemm, P. (2001) Valency conversion in the type 1 fimbrial adhesin of Escherichia coli. Mol Microbiol 41: 675–686.

Springall, T., Sheerin, N.S., Abe, K., Holers, V.M., Wan, H., and Sacks, S.H. (2001) Epithelial secretion of C3 promotes colonization of the upper urinary tract by Escherichia coli. Nat Med 7: 801–806.

Steadman, R., Topley, N., Jenner, D.E., Davies, M., and Williams, J.D. (1988) Type 1 fimbriate Escherichia coli stimulates a unique pattern of degranulation by human polymorphonuclear leukocytes. Infect Immun 56: 815–822.

Stromberg, N., Marklund, B.I., Lund, B., Ilver, D., Hamers, A., Gaastra, W., et al. (1990) Host-specificity of uropathogenic Escherichia coli depends on differences in binding specificity to Gal(1–4) Gal-containing isoreceptors. EMBO J 9: 2001–2010.

Stromberg, N., Nyholm, P.-G., Pascher, I., and Normark, S. (1991) Saccharide orientation at the cell surface affects glycolipid receptor function. Proc Natl Acad Sci USA 88: 9340–9344.

Sun, T.T., Zhao, H., Provet, J., Aebi, U., and Wu, X.R. (1996) Formation of asymmetric unit membrane during urothelial differentiation. Mol Biol Report 23: 3–11.

Sung, M., Fleming, K., Chen, H.A., and Matthews, S. (2001) The solution structure of PapGII from uropathogenic Escherichia coli and its recognition of glycolipid receptors. EMBO Rep 2: 621–627.

Svanborg, C., Frendeus, B., Godaly, G., Hang, L., Hedlund, M., and Wachtler, C. (2001) Toll-like receptor signaling and chemokine receptor expression influence the severity of urinary tract infection. J Infect Dis 183 (Suppl. 1): S61–S65.

Svanborg, C., and Godaly, G. (1997) Bacterial virulence in urinary tract infection. Infect Dis Clin North Am 11: 513–529.

Svanborg, C., Hedlund, M., Conelli, H., Agace, W., Duan, R.D., Nilsson, A., and Wullt, B. (1996) Bacterial adherence and...
mucosal cytokine responses. Receptors and transmembrane signaling. *Ann N Y Acad Sci* **797**: 177–190.

Tewari, R., MacGregor, J.I., Ikeda, T., Little, J.R., Hultgren, S.J., and Abraham, S.N. (1993) Neutrophil activation by nascent FimH subunits of type 1 fimbriae purified from the periplasm of *Escherichia coli*. *J Biol Chem* **268**: 3009–3015.

Thankavel, K., Madison, B., Ikeda, T., Malaviya, R., Shah, A.H., Arumugam, P.M., and Abraham, S.N. (1997) Localization of a domain in the FimH adhesin of *Escherichia coli* type 1 fimbriae capable of receptor recognition and use of a domain-specific antibody to confer protection against experimental urinary tract infection. *J Clin Invest* **100**: 1123–1136.

Tseng, C.C., Huang, J.J., Ko, W.C., Yan, J.J., and Wu, J.J. (2001) Decreased predominance of papG class II allele in *Escherichia coli* strains isolated from adults with acute pyelonephritis and urinary tract abnormalities. *J Urol* **166**: 1643–1646.

Uhlen, P., Laestadius, A., Jahnukainen, T., Soderblom, T., Backhed, F., Celsi, G., *et al.* (2000) Alpha-haemolysin of uropathogenic *E. coli* induces Ca2+ oscillations in renal epithelial cells. *Nature* **405**: 694–697.

Warren, J.W., Mobley, H.L., and Trifillis, A.L. (1988) Internalization of *Escherichia coli* into human renal tubular epithelial cells. *J Infect Dis* **158**: 221–223.

van der Woude, M., Braaten, B., and Low, D. (1996) Epigenetic phase variation of the pap operon in *Escherichia coli*. *Trends Microbiol* **4**: 5–9.

Wullt, B., Bergsten, G., Connell, H., Rollano, P., Gebretsadik, N., Hull, R., and Svanborg, C. (2000) P fimbriae enhance the early establishment of *Escherichia coli* in the human urinary tract. *Mol Microbiol* **38**: 456–464.

Xia, Y., Gally, D., Forsman-Semb, K., and Uhlin, B.E. (2000) Regulatory cross-talk between adhesin operons in *Escherichia coli*: inhibition of type 1 fimbriae expression by the PapB protein. *EMBO J* **19**: 1450–1457.

Zhang, L., Foxman, B., Manning, S.D., Tallman, P., and Marrs, C.F. (2000) Molecular epidemiologic approaches to urinary tract infection gene discovery in uropathogenic *Escherichia coli*. *Infect Immun* **68**: 2009–2015.

Zhou, G., Mo, W.J., Sebbel, P., Min, G., Neubert, T.A., Glockshuber, R., *et al.* (2001) Uroplakin Ia is the urothelial receptor for uropathogenic *Escherichia coli*: evidence from in vitro FimH binding. *J Cell Sci* **114**: 4095–4103.