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Structure, Function, and Assembly of Adhesive Organelles by Uropathogenic Bacteria

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ABSTRACT Bacteria assemble a wide range of adhesive proteins, termed adhesins, to mediate binding to receptors and colonization of surfaces. For pathogenic bacteria, adhesins are critical for early stages of infection, allowing the bacteria to initiate contact with host cells, colonize different tissues, and establish a foothold within the host. The adhesins expressed by a pathogen are also critical for bacterial-bacterial interactions and the formation of bacterial communities, including biofilms. The ability to adhere to host tissues is particularly important for bacteria that colonize sites such as the urinary tract, where the flow of urine functions to maintain sterility by washing away non-adherent pathogens. Adhesins vary from monomeric proteins that are directly anchored to the bacterial surface to polymeric, hair-like fibers that extend out from the cell surface. These latter fibers are termed pili or fimbriae, and were among the first identified virulence factors of uropathogenic *Escherichia coli*. Studies since then have identified a range of both pilus and non-pilus adhesins that contribute to bacterial colonization of the urinary tract, and have revealed molecular details of the structures, assembly pathways, and functions of these adhesive organelles. In this review, we describe the different types of adhesins expressed by both Gram-negative and Gram-positive uropathogens, what is known about their structures, how they are assembled on the bacterial surface, and the functions of specific adhesins in the pathogenesis of urinary tract infections.

INTRODUCTION

Bacteria assemble a variety of adhesive proteins (adhesins) on their surface to mediate binding to receptors and colonization of surfaces. For pathogenic bacteria, adhesins are critical for early stages of infection, allowing the bacteria to initiate contact with host cells, colonize different tissues, and establish a foothold within the host. Adhesins recognize specific receptors expressed by specific subsets of host cells. Therefore, the repertoire of adhesins expressed by a pathogen play a major role in dictating the tropism of the pathogen toward specific host tissues and organs. Moreover, binding of bacterial adhesins to host cell receptors influences subsequent events by triggering signaling pathways in both the host and bacterial cells. These signaling pathways may determine whether the bacteria remain extracellular or become internalized, and influence the intracellular trafficking of invaded bacteria and their ability to survive and replicate (1, 2). The adhesins expressed by a pathogen are also critical for bacterial-bacterial interactions and the formation of bacterial communities, including biofilms. The ability to adhere to host tissues is particularly important for bacteria that colonize sites that include the urinary tract, where the flow of urine functions to maintain sterility by washing away non-adherent pathogens.

Adhesins vary from monomeric proteins that are directly anchored to the bacterial surface to polymeric, hairlike fibers that extend out from the cell surface. These latter fibers are termed pili or fimbriae, and were

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among the first identified virulence factors of uropathogenic *Escherichia coli* (UPEC) (3). Pili were first described in the late 1940s and early 1950s as bacterial surface structures distinct from flagella (4). Duguid et al. used the term *fimbriae*, Latin for thread or fiber, to describe surface appendages that allowed *E. coli* to bind to and agglutinate erythrocytes (5). Brinton later used the term *pili*, Latin for hair, to describe the non-flagellar surface structures expressed by *E. coli* (6). Ottow subsequently proposed that the term pili be reserved for the F or conjugative pili involved in bacterial mating, and that the term fimbriae should be used to describe surface fibers involved in adhesion (4). However, today the terms pili and fimbriae are generally used interchangeably. We will refer to these structures collectively as pili.

Various schemes have been proposed to classify the different types of pili (4, 7–10). Although most of these classification schemes are no longer in common use, parts have entered the standard nomenclature. Pili were originally classified as mannose resistant (MR) or mannose sensitive (MS), based on their ability to agglutinate erythrocytes in the presence or absence of mannosides (11, 12). This classification led to the term type 1 pili, which is still in current use, to refer to MS surface fibers. The MR pili were initially divided into the P and unknown (X) pili, with the unknown pili now defined to include the S, Dr, and additional pilus adhesins (3). Uropathogenic bacteria have been closely associated with the discovery and characterization of pili. The chromosomal gene clusters responsible for expression of both type 1 and P pili were first cloned from the J96 UPEC strain (13), and the genes coding for S pili were isolated from UPEC strain 536 (14). As discussed in detail in the following section, much of our current understanding of the structure, assembly, and functions of bacterial pili stems from studies of the type 1 and P pili originally isolated from UPEC.

Bacteria are now known to express a number of different types of pilus structures and other non-flagellar surface appendages (15). One additional structure, curli, is expressed by UPEC and imparts unique characteristics to the bacteria that influence colonization within the urinary tract, including promoting biofilm formation (16). Curli are assembled by a completely different mechanism from pili such as the type 1 and P pili, and appear as aggregated masses on the bacterial surface rather than hair-like fibers. Pilus assembly is not restricted to Gram-negative bacteria. Pili were observed on the Gram-positive bacterium *Corynebacterium renale* in the 1960s (17, 18), but this observation was largely forgotten until studies dating from 2003 by Ton-That and Scheewind to characterize pilus biogenesis in *Corynebacterium diphtheriae* (19, 20). A number of different Gram-positive bacteria are now known to assemble adhesive pili associated with virulence and this is an active area of research. The Gram-positive pili have unique structural features and assembly mechanisms compared to Gram-negative pili (21, 22).

Pili and other extended surface fibers increase the functional reach of adhesins, enabling the bacteria to act at a distance. Pili place adhesins outside capsular or other protective surface structures, allowing contact with receptors while maintaining the protective integrity of the bacterial envelope. The ability to initiate contact at a distance also provides a means for pathogenic bacteria to avoid detection or uptake by host cells. Despite these advantages of pilus adhesins, bacteria also express a range of non-pilus adhesins, which are anchored directly on the bacterial surface. Non-pilus adhesins confer intimate binding to surfaces and are often associated with formation of bacterial colonies and biofilms. Gram-negative uropathogens display several adhesins important for pathogenesis on their outer membrane, with the majority of these adhesins assembled by the autotransporter (type V) secretion pathway. Gram-positive uropathogens also display adhesins on their surface important for colonizing the urinary tract. These Gram-positive adhesins typically are covalently linked to the peptidoglycan cell wall and are termed MSCRAMMs (microbial surface components recognizing adhesive matrix molecules) (22, 23).

Table 1 lists adhesins that contribute to infection of the urinary tract by Gram-negative and Gram-positive uropathogens. In this chapter, we will describe the different types of adhesins, what is known about their structures, and how they are assembled on the bacterial surface. We will also describe the functions of specific adhesins in the pathogenesis of urinary tract infections (UTIs). For the Gram-negative adhesins, we will focus our description on UPEC, which serves as a model system and for which extensive studies have been done.

ADHESINS EXPRESSED BY GRAM-NEGATIVE UROPATHOGENS

Pili Assembled by the Chaperone/Usher Pathway

A wide range of Gram-negative bacteria use the chaperone/usher (CU) pathway to assemble a superfamily of virulence-associated adhesive surface fibers (24–27). The CU pathway takes its name from the components of its

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TABLE 1 Adhesins of uropathogenic bacteria

Organism	Assembly pathway	Adhesin	Associated UTI disease or function	Receptor(s)	Reference(s)
GRAM-NEGATIVE BACTERIA					
Escherichia coli					
	Chaperone/usher pathway				
		P pili	Pyelonephritis	Digalactose (galabiose)	165, 365, 366
		Type 1 pili	Cystitis	Mannosylated proteins, uroplakin, β_1 and α_3 integrin	34, 141, 367
		S pili	Ascending UTI	α-sialic acid	116, 368, 369
		F1C pili	Ascending UTI	galactosylceramide on bladder epithelium and globotriaosylceramide on kidney epithelium	128, 370
		Afa/Dr pili	Recurrent/chronic UTI, cystitis, pyelonephritis	Dr[a], DAF, type IV collagen, $\alpha_5\beta_1$ integrin, CEACAM family proteins	51, 114, 123
		Yad pili	Ascending UTI	Bladder epithelial cells	43, 118
		Ygi pili	Ascending UTI	Human embryonic kidney cells	118
		F9 pili	Biofilm formation	?	120
		Auf pili	?	?	119
		Type 3 pili	CAUTI	?	371
	Autotransporter				
		Ag43	UTI persistence, biofilm formation	Collagen, laminin	263–265
		UpaB	?	Fibronectin, fibrinogen, laminin	270
		UpaC	Biofilm formation	?	270
		UpaH	Biofilm formation, bladder colonization	Collagen V, laminin, fibronectin	217, 272
		UpaG	Biofilm formation	Fibronectin, laminin	232
		FdeC	Bladder and kidney colonization	?	273, 274
	Outer membrane protein				
		Iha	Fitness in urinary tract	?	278, 279
	Type I secretion				
		TosA	Fitness in urinary tract	Kidney epithelium	284, 287
	Extracellular nucleation/precipitation				
		Curli	Biofilm formation, ?	Fibronectin, laminin, H-kininogen, fibrinogen, factor XII	174, 178, 196
Klebsiella pneumoniae					
	Chaperone/usher pathway				
		Type 1 pili	Ascending UTI, CAUTI	Mannosylated proteins	372–374
		Type 3 (MR/K) pili	CAUTI	Type V collagen	373, 375, 376
Citrobacter freundii					
	Chaperone/usher pathway				
		Type 3 pili	CAUTI	?	377
Proteus mirabilis					
	Chaperone/usher pathway				
		MR/P	Pyelonephritis, ascending UTI	Mannose-resistant	378–380
		PMF (MR/K)	Ascending UTI	?	381, 382
		UCA (NAF)	Colonization of urinary tract, complicated UTI	GalNAcβ(1-4)Gal	383–385
		ATF	?	?	386, 387
	Autotransporter				
		AipA	Bladder and kidney colonization	Collagen I, collagen IV, laminin	220
		TaaP	Bladder colonization	Collagen I, collagen IV, laminin	220
GRAM-POSITIVE BACTERIA					
Staphylococcus saprophyticus					
	Sortase-assembled MSCRAMM				
		UafA	Ascending UTI	?	307, 327
		UafB	Ascending UTI	Fibronectin, fibrinogen	308
		SdrI	UTI persistence	Fibronectin	296, 309, 329
Enterococcus faecalis					
	Sortase-assembled MSCRAMM				
		Ace	CAUTI, ascending UTI	Collagen I and IV	297, 306, 312

(continued)

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TABLE 1 Adhesins of uropathogenic bacteria

Organism	Assembly pathway	Adhesin	Associated UTI disease or function	Receptor(s)	Reference(s)
	Sortase-assembled pili				
		Ebp	CAUTI, ascending UTI	?	340, 345, 352
	Unknown				
		EfbA	Ascending UTI	Fibronectin	331
		Esp	Urinary tract colonization and persistence, biofilm formation	?	332, 333
Enterococcus faecium					
	Sortase-assembled pili				
		Ebp_{fm}	CAUTI, ascending UTI	?	339, 347

secretion machinery, which consist of a dedicated periplasmic chaperone and an integral outer membrane protein termed the usher. The CU pathway builds a diverse array of peritrichous surface fibers, ranging from thin, flexible filaments to rigid, rod-like organelles. For uropathogenic bacteria, pili assembled by the CU pathway mediate adhesion to receptors in the urinary tract, initiating infection and promoting bacterial colonization. Pili are critical virulence factors of uropathogenic bacteria and have been the subject of intense study (Table 1). The CU pili expressed by uropathogenic bacteria are exquisitely adapted to colonization within the urinary tract, engineered to withstand and take advantage of forces encountered during colonization such as the flow of urine (28–31). In addition to binding to host molecules, CU pili are important for bacterial-bacterial interactions, biofilm formation, and adhesion to abiotic surfaces. Moreover, binding of bacteria to host cells via CU pili modulates host–signaling pathways and promotes subsequent stages of pathogenesis, including invasion inside host cells (32–38).

Genes coding for CU pili are found on both the bacterial chromosome and on plasmids, and are clustered together with a similar organization: a 5′ regulatory region that is followed by a single downstream operon encoding the required pilus structural proteins and assembly components (Fig. 1). CU gene clusters are often associated together with other virulence determinants in pathogenicity islands, which have characteristics indicating acquisition by horizontal gene transfer (39). A single bacterial genome often contains multiple CU pathways, which presumably provides the ability to adhere to a variety of different receptors and surfaces (40–42). A recent genomic analysis found that *E. coli* strains encode as many as 17 CU gene clusters and that UPEC strains encode from 9 to 12 intact CU gene clusters (43). Many of the CU gene clusters present in a bacterial genome are not expressed under laboratory growth conditions and their functions remain unknown (41). The expression of CU gene clusters is typically highly regulated, subject to phase variation, and responsive to environmental cues (44, 45). Regulatory cross talk may occur among different CU gene clusters (46–48). This cross talk likely ensures that a given bacterium only expresses a single pilus at a given time, thus controlling adhesive specificity. Furthermore, expression of adhesive pili has been shown to be inversely correlated with the expression of flagella for motility (49).

Much of what we know about the biogenesis and functions of CU pili comes from work on the prototypical type 1 and P pili expressed by UPEC, which bind to receptors in the bladder and kidney, respectively. We will focus on these pili as models, but will also discuss additional CU pili identified as important for pathogenesis in the urinary tract. Although we will limit this discussion to pili expressed by UPEC, CU pili have been identified as virulence factors in other uropathogens, particularly for *Proteus mirabilis* (Table 1) (50).

Structure of CU pili

The pilus fiber

Pili assembled by the CU pathway range from 2 nanometer (nm) to 10 nm in diameter and generally 1 micrometer (μm) to 3 μm in length. The pili are linear fibers built from thousands of copies of non-covalently interacting subunit proteins, termed pilins. Some pili adopt a final helical quaternary structure, resulting in the formation of rigid, rod-like organelles. Alternatively, the pili may remain as linear, flexible fibers, which, in some cases, form amorphous or 'afimbrial' structures. Many pili assembled by the CU pathway are composite structures containing both a rigid, helical rod, which extends out from the bacterial surface, as well as a thin, flexible tip structure, which is located at the distal end of the rod and contains the adhesive activity. Type 1 and

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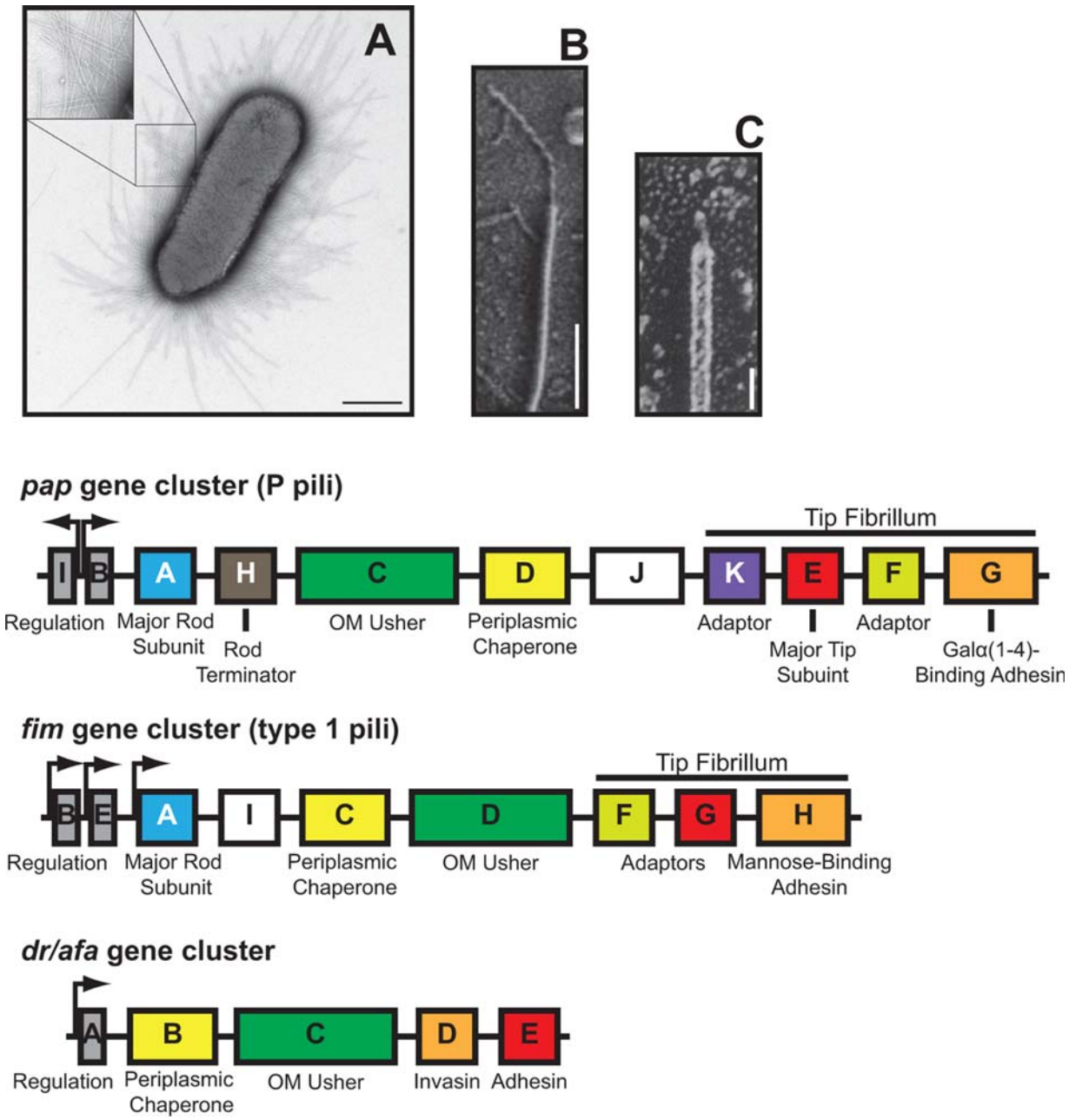

FIGURE 1 Representative CU gene clusters and pili. Gene clusters coding for P (*pap*), type 1 (*fim*) and Dr/Afa pili are depicted, with the functions of the genes indicated. Electron micrographs are shown for (A) an *E. coli* bacterium expressing type 1 pili, (B) a P pilus fiber, and (C) a type 1 pilus fiber. Scale bars equal 700 nm (A), 100 nm (B), and 20 nm (C). The images in panels A-C are reprinted from references 138, 157, and 137, respectively, with permission of the publishers. doi:10.1128/microbiolspec.UTI-0018-2013.f1

P pili expressed by UPEC are prototypical composite organelles with distinct rod and tip structures (Fig. 1). The Afa/Dr family of pili expressed by UPEC and other pathogenic *E. coli* are well-studied examples of thin, flexible fibers that often have an amorphous appearance by electron microscopy (51).

The structures of pilins and many aspects of pilus assembly by the CU pathway are understood in atomic detail (26, 52–57). All pilins contain an immunoglobulin (Ig)-like fold termed the pilin domain (Fig. 2). Canonical Ig folds comprise seven β-strands arranged into two sheets as a β-sandwich (58). However, pilins lack

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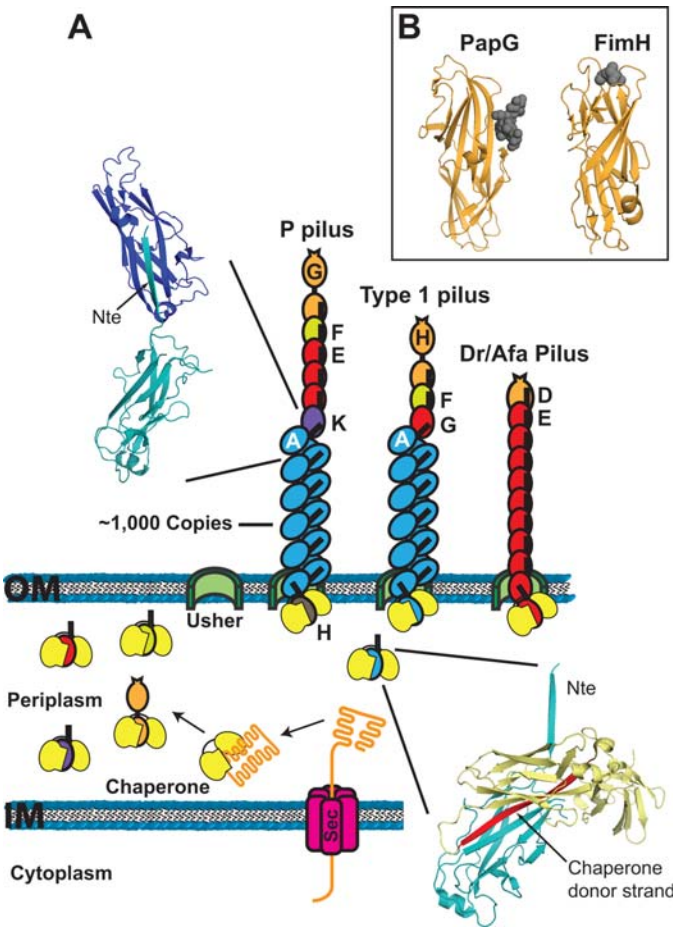

FIGURE 2 **(A)** Model for pilus biogenesis by the CU pathway. Pilus subunits enter the periplasm as unfolded polypeptides via the Sec system. Subunits fold upon forming binary complexes with the periplasmic chaperone (yellow). The crystal structure in the lower right depicts the chaperone-subunit donor strand exchange reaction (PapD-PapA; PDB ID: 2UY6), with the chaperone donor strand indicated in red. Pilus assembly takes place at the outer membrane usher, which catalyzes the exchange of chaperone-subunit for subunit-subunit interactions. Models for assembled P, type 1 and Afa/Dr pilus fibers are shown. The crystal structure in the upper left depicts the subunit-subunit donor strand exchange reaction that occurs in the pilus fiber (PapA-PapA; PDB ID: 2UY6), with the Nte donor strand indicated. **(B)** Crystal structures of the PapG (P pili; PDB ID: 1J8R) and FimH (type 1 pili; PDB ID: 1KLF) adhesin domains with bound globoside and mannose, respectively. The sugars are depicted as dark gray spheres. doi:10.1128/microbiolspec.UTI-0018-2013.f2

the seventh, C-terminal β-strand (the G strand) and thus are unable to complete their own fold (52–55). This missing strand results in a deep groove on the surface of the subunit, exposing its hydrophobic core. To complete their folds, pilins rely on structural information provided by interaction with the periplasmic chaperone or with neighboring subunits in the pilus fiber.

Subunit-subunit interactions in the pilus fiber are mediated by a mechanism termed donor strand exchange (54, 55). Pilins contain a conserved N-terminal extension (Nte) in addition to the pilin domain. In the pilus fiber, the Nte of one pilus subunit is 'donated' to the preceding subunit, completing the Ig fold of the preceding subunit (Fig. 2). Therefore, the pilus fiber consists of an array of Ig folds, with each subunit noncovalently bound to the preceding subunit by donor strand exchange. This arrangement provides great mechanical strength and stability to the pili, which is reflected by the property that subunit–subunit interactions in the pilus are resistant to dissociation by heat and denaturants (59, 60). A high level of mechanical strength is essential for the pili to maintain adhesion in the face of shear forces encountered from the flow of urine. The helical pilus rod provides an additional mechanism to withstand hydrodynamic forces in the urinary tract; the helical rod is able to uncoil under stress to an extended linear fiber, thereby acting as a spring or shock absorber to prevent breakage of the pilus and extend the lifetime of pilus-receptor interactions (28, 29, 31, 61).

The pilus adhesin

The receptor-binding activity of pili is conferred by the pilus adhesin. For composite pili such as type 1 and P pili, the adhesin is located in single copy at the distal end of the tip fiber (Fig. 2). Such pili have been termed monoadhesive pili (25). In contrast, for pili lacking a district tip structure, the main structural subunit that builds the pilus fiber may also contain receptor-binding sites along exposed surfaces and thus the entire pilus may function in adhesion (57, 62–64). Such fibers are termed polyadhesive pili. Afa/Dr pili are polyadhesive fibers with a single major structural subunit/adhesin; however, these pili also have a separate subunit, termed the invasin, with distinct binding activity and which promotes uptake inside host cells (38, 64, 65). The invasin subunit is present in single copy at the distal end of the pilus fiber (Fig. 2).

Crystal structures have been solved for several adhesins from monoadhesive pili (52, 66–71). In contrast to other pilus subunits, the adhesins are two domain proteins, containing an N-terminal receptor-binding or adhesin domain (in place of the Nte) and a C-terminal pilin domain. The pilin domain mediates incorporation of the adhesin into the pilus fiber and is an incomplete Ig-like fold as found for all CU pilins. Adhesin domains

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also have Ig-like folds, but the folds are complete (not lacking the terminal β-strand) and structurally distinct from the pilin domain. Despite their common architecture, adhesins vary greatly in sequence and employ distinct receptor binding mechanisms, reflecting their specific functions within the host (72). The FimH adhesin from type 1 pili folds as an elongated 11-stranded β-barrel with a jelly roll-like topology (52, 67). The binding site for the mannose ligand is located at the tip of the adhesin domain and is formed by a deep, negatively-charged pocket surrounded by a hydrophobic ridge (Fig. 2B). In comparison, the adhesin domain of the P pilus adhesion, PapG, adopts a structure with two sub-regions; one region having a β-barrel fold similar to FimH and the other region having a unique, largely β-sheet structure that contains the binding site for the globoside receptor (66, 68). In contrast to FimH, the receptor-binding site of PapG is located in a shallow groove along the side of the adhesin (Fig. 2B). For polyadhesive fibers such as Afa/Dr pili, both the main structural subunit and the tip-located invasin function as adhesins and both are single domain proteins with Ig-like pilin domains (57, 62, 65, 73). However, the invasin subunit lacks an Nte donor strand, thus restricting its position to the tip of the fiber. For the AfaD and DraD major subunits, distinct receptor-binding sites for CD55/decay accelerating factor (DAF) and for members of the carcinoembryonic antigen family (CEACAM) have been located along opposite sides of the pilin domain (25, 57, 62). These binding sites would be repetitively presented along the length of the assembled fiber.

Pilus adhesins such as FimH exhibit the property of shear-enhanced binding, which enables tighter binding under conditions of shear stress, including the shear stress encountered during bacterial colonization of the urinary tract (30). The application of shear stress causes FimH to switch from a low-affinity to a high-affinity binding state. This greater affinity presumably allows the bacteria to avoid being washed away by the flow of urine, and may also provide a mechanism for the bacteria to discriminate between surface-located and soluble receptors, as binding to the latter will not result in force generation on the pilus and FimH will stay in the low-affinity state. The shear-enhanced binding of FimH is mediated by a catch-bond mechanism that involves allosteric activation of the adhesin domain (74). When incorporated into the type 1 pilus tip fiber, the pilin domain of FimH interacts with the adhesin domain, causing structural alterations of the adhesin that weaken its mannose binding pocket. However, the application of force to the pilus fiber causes the FimH pilin and adhesin domains to separate, allowing the binding pocket to clamp tightly around its mannose ligand (75). Moreover, the physical properties of both the type 1 pilus tip fiber and the helical pilus rod appear to be designed to optimize the shear-enhanced behavior of FimH, and the flexibility of the pilus tip likely provides FimH maximum opportunity to find its target receptors (76, 77).

Pilus Assembly by the Chaperone/Usher Pathway

Formation of chaperone-subunit complexes in the periplasm

Pilus subunits are synthesized with an N-terminal signal sequence that directs them to the Sec general secretory pathway for translocation to the periplasm (78). The signal sequence is cleaved in the periplasm, and the subunits form stable, binary complexes with the periplasmic chaperone (Fig. 2A). The chaperone enables proper folding of the pilus subunits, prevents premature subunit-subunit interactions, and maintains the subunits in an assembly-competent state (52–55). In the absence of the chaperone, pilus subunits misfold and form aggregates that are degraded by the DegP periplasmic protease (79, 80).

The structure of the PapD chaperone and subsequent structures of chaperone-subunit complexes revealed the molecular basis for chaperone function in pilus biogenesis (52–54, 81–83). As described above, pilins have an incomplete Ig fold, lacking the C-terminal G β-strand. The chaperone contains two Ig-like domains oriented in an L or boomerang shape. The binding site for subunits resides in the cleft between the two domains and extends out along the chaperone's N-terminal domain (domain 1). The chaperone functions by a mechanism termed donor strand complementation, in which the chaperone inserts its G1 β-strand and a portion of its F1-G1 loop into the groove caused by the missing G strand of the subunit, completing the Ig fold of the pilin domain (Fig. 2A) (52, 53, 83, 84). Conserved sequence differences in the F1–G1 loop region of chaperones defines two subfamilies of CU pathways: chaperones with a short F1–G1 loop belong to the FGS (F1–G1 short) subfamily and chaperones with a long F1–G1 loop belong to the FGL subfamily (64, 85). Interestingly, these differences in the chaperones correlate with differences in the types of surface fibers assembled. FGL chaperones assemble only thin or amorphous pili comprising only one or two types of pilins (e.g., Afa/Dr pili), whereas FGS chaperones assemble both rod-like and thin pilus fibers that generally comprise multiple different pilins

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and may have composite architectures (e.g., type 1 and P pili).

The groove in the pilin domain caused by the missing β-strand contains a series of binding pockets, termed P1–5 (54). The G1 β-strand donated by the chaperone contains a conserved motif of alternating hydrophobic residues, and during donor strand exchange these residues insert into the P1–4 pockets of the subunit, forming a β-zipper interaction (55, 86). In FGL chaperones, the longer G1 donor strand fills the P5 binding pocket as well, but this interaction is weaker than at the other pockets (86, 87). The chaperone G1 β-strand is inserted parallel to the F strand of the subunit, forming a non-canonical Ig fold. This, together with the large size of the residues inserted by the chaperone, maintains pilins in an open, "activated" state, which enables subsequent assembly into the pilus fiber (54, 55, 87). The groove of the pilin domain is also the site of subunit-subunit interactions, which are mediated by the donor strand exchange reaction as described above for the pilus fiber (54, 55). Thus, donor strand complementation by the chaperone couples the folding of pilins with the simultaneous capping of their interactive surfaces, preventing premature fiber assembly in the periplasm. Recent studies have shown that chaperones also perform a quality control function during the initial binding of pilus subunits and that formation of chaperone-subunit complexes results in an allosteric change in the chaperone that permits binding to the outer membrane usher assembly platform (88, 89).

Assembly of the pilus fiber at the outer membrane

Chaperone-subunit complexes must interact with the outer membrane usher for release of the chaperone, assembly of subunits into the pilus fiber, and secretion of the fiber to the cell surface through the usher channel (56, 90). The usher acts as a pilus assembly catalyst, accelerating the rate of subunit incorporation into the pilus fiber (91). Subunit-subunit interactions form at the periplasmic face of the usher via the donor strand exchange mechanism (54, 55). The donated subunit Nte contains a conserved motif of alternating hydrophobic residues, similar to the chaperone G1 β-strand (92, 93). At the usher, the hydrophobic residues of the Nte from an incoming chaperone-subunit complex insert into the subunit groove of the preceding chaperone-subunit complex bound at the usher, displacing the donated G1 β-strand of the chaperone from the preceding subunit by a concerted strand displacement mechanism that initiates at the P5 pocket (54, 55, 86, 94, 95). In contrast to the donated chaperone β-strand, the Nte is inserted anti-parallel to the F strand of the preceding subunit and inserts smaller-sized residues into the subunit groove, thus completing the Ig fold of the pilin domain in a canonical fashion and allowing the subunit to adopt a highly stable final state (54, 55, 60, 87). ATP is not available in the periplasm and pilus biogenesis at the outer membrane usher does not require input from other energy sources (96, 97). The canonical Ig fold formed by donor strand exchange represents a more compact, lower energy state compared to the non-canonical Ig fold formed by donor strand complementation with the chaperone (54, 55, 87). This topological transition from the higher-energy chaperone-subunit complex to the lower-energy subunit-subunit interaction provides the driving force for fiber formation and secretion at the usher (98).

Pili are assembled in a top-down order, with the adhesin incorporated first, followed by the rest of the pilus tip and finally the rod. Each subunit specifically interacts with its appropriate neighbor subunit in the pilus, with the specificity of binding determined by the donor strand exchange reaction (99–101). In addition, the usher ensures ordered and complete pilus assembly by differentially recognizing chaperone-subunit complexes according to their final position in the pilus; i.e., chaperone-adhesin complexes have highest affinity for the usher, whereas chaperone-rod subunit complexes have low affinity (102–104). The usher channel is only wide enough to allow secretion of a linear fiber of folded pilus subunits (56, 90). Therefore, the pilus rod is constrained to a linear fiber as it passes through the usher and only converts to its final helical form upon reaching the bacterial surface.

The pilus usher

Ushers are large, integral outer-membrane proteins containing five domains: a central transmembrane β-barrel domain that forms the secretion channel, a middle domain located within the β-barrel region that forms a channel gate (the plug domain), a periplasmic N-terminal domain (NTD), and two periplasmic C-terminal domains (CTD1 and CTD2) (Fig. 3) (56, 90, 105–109). The NTD provides the initial binding site for chaperone-subunit complexes and functions in the recruitment of periplasmic complexes to the usher (105, 110). The CTDs provide a second binding site for chaperone-subunit complexes and anchor the growing pilus fiber (56). The usher is present as a dimeric complex in the OM, but only one channel is used for secretion of the pilus fiber and the function of the usher dimer remains

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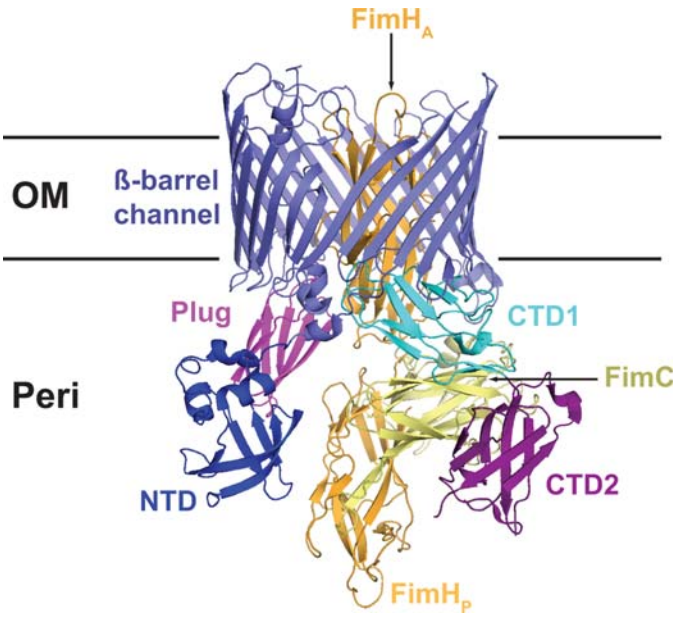

FIGURE 3 Crystal structure of the FimD-FimC-FimH type 1 pilus assembly intermediate (PDB ID: 3RFZ). The Usher NTD, plug, β-barrel channel, and CTD domains are indicated. The FimH adhesin domain (FimH$_A$) is inserted inside the usher channel, and the FimH pilin domain (FimH$_P$) and bound FimC chaperone are located at the usher CTDs. doi:10.1128/microbiolspec.UTI-0018-2013.f3

to be determined, particularly since the usher monomer appears to be sufficient for pilus assembly (56, 90, 106, 111, 112).

The structure of the type 1 pilus usher FimD bound to the FimC-FimH chaperone-adhesin complex was recently solved, revealing the usher pilus assembly machine in action (Fig. 3) (56). The usher channel is formed by a 24-stranded β-barrel that is occluded by an internal plug domain (56, 90). The binding of the FimH adhesin to FimD activates the usher for pilus biogenesis (91, 103, 113), resulting in displacement of the plug to the periplasm and insertion of the FimH-adhesin domain inside the usher channel. The FimH-pilin domain remains in complex with the FimC chaperone and bound to the usher CTDs (Fig. 3). CU pili extend by step-wise addition of new chaperone-subunit complexes to the base of the fiber. New chaperone-subunit complexes are recruited by binding to the usher NTD, which is unoccupied in the FimD–FimC–FimH structure (56, 105, 110). Modelling studies suggest that binding of a chaperone-subunit complex to the usher NTD would perfectly position the Nte of the newly recruited subunit to initiate donor strand exchange with the P5 pocket of the subunit bound at the usher CTDs, providing a molecular explanation for the catalytic activity of the usher in pilus assembly (56). Following donor strand exchange, the chaperone is displaced from the subunit bound at the CTDs and released into the periplasm. To reset the usher for another round of subunit incorporation, the newly incorporated chaperone-subunit complex must transfer from the NTD to the CTDs, concomitant with translocation of the pilus fiber through the usher channel toward the cell surface. Repeated iterations of this cycle would then result in assembly and secretion of a complete pilus fiber.

Functions of CU Pili Expressed by UPEC

A number of different CU pili have been identified that contribute to colonization of the urinary tract by UPEC (Table 1). In addition to type 1 and P pili, which are described in detail in the following paragraphs, CU systems with demonstrated or putative roles in UTIs include Afa/Dr, S, F1C, F9, type 3, Auf, Yad and Ygi pili (51, 114–120). Further characterization is needed for many of these systems, which may have roles in direct adherence to host receptors or may facilitate bacterial-bacterial interactions and biofilm formation. The best characterized of these additional CU pili are the Afa/Dr family, which includes Dr, F1845, Afa, Nfa, and Aaf pili. Afa/Dr pili are thin, polyadhesive fibers that are expressed by diffusely adhering strains of diarrheagenic *E. coil* (DAEC) in addition to being prominent virulence factors of UPEC (51, 114, 121). Afa/Dr adhesins bind to the Dr^a blood group antigen and have affinity for DAF, members of the CEACAM family, type IV collagen, and $\alpha_5\beta_1$ integrin (122–124). In contrast to the Afa/Dr polyadhesins, S and F1C pili are structurally similar to type 1 and P pili. S pili bind to sialyl-galactoside moieties on extracellular matrix proteins, such as fibronectin and laminin (125, 126). S pili are expressed by clinical UPEC isolates and expression of S pili confers binding to bladder and kidney epithelial cells, indicating potential roles in ascending UTIs (115, 127). F1C pili have affinity for globotriaosylceramide, which is present on the kidney epithelium, and for galactosylceramide, found in the bladder, kidney, and ureters (128).

Type 1 pili

Type 1 pili are expressed by most strains of *E. coli* and mediate binding to a variety of surfaces and host tissues in a mannose-sensitive manner. Type 1 pili are a major virulence factor of UPEC and antibodies to the type 1 pilus adhesin FimH provide protection against urinary tract infection by *E. coli* in both murine and primate models (129, 130). However, a definitive requirement for type 1 pili in human UTIs has remained elusive (131),

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likely due to the large repertoire of adhesins expressed by uropathogenic strains. UPEC use type 1 pili to bind to α-D-mannosylated proteins present in the bladder, leading to bacterial colonization, bladder epithelial cell invasion, and the development of cystitis (33, 132). In addition to urothelial cells, type 1 pili have been reported to bind to Tamm-Horsfall protein, surface glycoproteins of immune cells, extracellular matrix proteins, and abiotic surfaces (133–136).

Type 1 pili are encoded by the *fim* gene cluster (Fig. 1), which is present on the chromosome of pathogenic as well as non-pathogenic and laboratory strains of *E. coli*. Type 1 pili are built from 4 different types of pilins arranged into a rigid helical rod measuring 6.9 nm in diameter, and a short tip fiber measuring 2 nm in diameter and generally 10 nm to 19 nm in length (Fig. 1) (137, 138). The type 1 pilus rod is built from more than 1,000 copies of the FimA major pilin arranged into a right-handed helix (138). Type 1 pilus tips contain a single copy of the FimH adhesin at the distal end, followed by the FimG and FimF adaptor subunits, which are generally present in single copy (Fig. 2A) (75, 137, 138). The mannose binding site of the FimH adhesin is located in a deep pocket at the tip of the adhesin domain (Fig. 2B) (67). This places the receptor-binding site at the most distal end of the type 1 pilus organelle, which presumably facilitates access of the pilus to its receptor.

Studies using the murine urinary tract infection model have revealed many aspects of type 1 pilus function during UPEC pathogenesis. On entering the urinary tract, UPEC use their type 1 pili to bind to uroplakins, mannosylated proteins that coat the luminal surface of the bladder, allowing the bacteria to colonize the bladder and avoid being washed out by the flow of urine (33, 139). Type 1 pili not only mediate binding of UPEC to the bladder surface, but also trigger host cell signaling pathways that lead to actin rearrangement in the urothelial cells and invasion of the bacteria inside the cells by a zipper-like mechanism (32, 33, 140). Additionally, bacterial uptake is facilitated by binding of type 1 pili to β1 and α3 integrins (34). Binding of *E. coli* to the urothelium leads to induction of innate host cell responses, including upregulation of proinflammatory cytokines and cell death pathways (33, 140, 141). The FimH adhesin acts as a pathogen-associated molecular pattern that is recognized by Toll-like receptor 4 (TLR4), present on bladder epithelial cells as well as macrophages, and stimulates immune signaling pathways through a mechanism independent of LPS (36).

Following uptake inside bladder epithelial cells, UPEC are initially contained within vesicles, which may be routed for exocytosis in a TLR4- and cyclic AMP-dependent mechanism that may be used by the host cells to expel the invading bacteria (142). Bacteria that evade expulsion enter the cytoplasm where they rapidly replicate to form aggregates termed intracellular biofilm-like communities or pods (143, 144). Bacteria within these intracellular communities are protected from innate host immune responses and shielded from antibiotics (145). Type 1 pili, which are known to contribute to the formation of extracellular biofilms (136), are also expressed by the intracellular bacteria and required for formation of the pods, separate from their function in host cell binding and invasion (35, 146). Urothelial cells respond to UPEC invasion by undergoing programmed cell death and exfoliating into the bladder lumen, a host defense mechanism to wash out the colonizing bacteria (33). However, UPEC counter this by fluxing out of the host cells and undergoing additional rounds of attachment to and invasion of neighboring cells, presumably mediated by type 1 pili as in the initial round of infection (144, 147). During this process, the *E. coli* may gain access to the underlying bladder epithelium, leading to the formation of quiescent bacterial reservoirs from which recurrent infections can be seeded to begin the infection process anew (147 , 148). Thus, type 1 pili function at multiple different points during UPEC pathogenesis in the urinary tract and have both extracellular and intracellular roles.

P pili

P pili are expressed by UPEC and are strongly associated with the ability of the bacteria to colonize the kidney and cause pyelonephritis (66, 149, 150). P pili bind to Gal(α1-4)Gal moieties present in the globoseries of glycolipids found in kidney epithelial cells. The glycolipid receptor is also part of the P blood group antigen, thus allowing P pilus-mediated agglutination of human erythrocytes (151). P pili are encoded by the chromosomal *pap* (pyelonephritis-associated pili) gene cluster (Fig. 1), which is present on pathogenicity islands of UPEC strains, and also found in *E. coli* causing neonatal meningitis and avian pathogenic strains (152). Individual *E. coli* strains may carry more than one *pap* gene cluster, located in different pathogenicity islands (153, 154). There are three predominant alleles of the P pilus adhesin PapG – class I, II, and III – which have specificities for receptor isotypes that differ in carbohydrate residues distal from the Gal(α1-4)Gal core (155, 156). Class II PapG is correlated with human kidney infections, whereas class III PapG is associated with colonization of the human bladder.

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P pili are built from six different structural subunits that form a right-handed helical rod and distal tip fiber, similar to type 1 pili (Fig. 1). The P pilus tip fiber is longer and more flexible compared to type 1 pilus tips, measuring approximately 40 nm in length. The P pilus tip is composed mainly of PapE, which is present at approximately 5 to 10 copies per pilus. The PapG adhesin is present in single copy at the distal end of the tip and is joined to PapE via the PapF adaptor subunit (157, 158) (Fig. 2A). Another adaptor subunit, PapK, links the tip fiber to the pilus rod (158). The helical P pilus rod measures 8.2 nm in diameter and is built from a linear homopolymer of over 1000 copies of the PapA major pilin (159). The P pilus rod is terminated by the PapH minor pilin, which also plays a role in anchoring the pilus fiber in the OM (160, 161).

The glycolipid binding site on the PapG adhesin is formed by a shallow pocket on one side of the adhesin domain (Fig. 2B) (66, 68). This is in contrast to the tip-located mannose-binding site of FimH on type 1 pili. P pili have a longer, more flexible tip fiber compared to type 1 pili. The flexibility of the P pilus tip and side-on orientation of the PapG binding site likely function in tandem to facilitate docking of the adhesin onto the globoside moiety of the glycolipid receptor, which is oriented parallel to the membrane surface (66).

Expression of P pili promotes ascending urinary tract infection and facilitates colonization of the kidneys by *E. coli* (150, 162). Consistent with a role in pathogenesis, vaccination with P pili was shown to provide protection against pyelonephritis in both murine and primate models (163, 164). However, studies using P pilus mutants have had variable results in establishing an essential requirement for the pili in kidney infections, likely due to the many different adhesins expressed by UPEC strains (165). As for type 1 pili, P pilus-mediated adhesion of UPEC to the urothelium stimulates cytokine production and resultant inflammatory responses in the urinary tract, which likely exacerbates kidney damage during acute pyelonephritis (37, 166, 167). Binding of P pili to its glycolipid receptor in kidney epithelial cells causes release of the second messenger ceramide, which forms the membrane anchor portion of the receptor. Ceramide is as an agonist for TLR4, and thus provides a potential link between bacterial adhesion and induction of innate immune pathways (168). PapG-mediated binding also activates signal transduction pathways within the bacteria (169). These pathways result in upregulation of iron acquisition systems and may prepare UPEC for colonization of the urinary tract.

Curli

Curli fibers, also called thin aggregative fimbriae, are produced by Gram-negative enteric bacteria such as *E. coli* and *Salmonella* and form part of a complex extracellular matrix that contributes to adhesion, biofilm formation, host colonization, and invasion (16, 170–172). The expression of curli imparts special properties to biofilm structures, allowing attachment to normally resistant surfaces such as Teflon and stainless steel (170). Curli were first characterized by Normark and colleagues as novel bacterial surface structures that conferred binding to fibronectin (173). Curli bind to range of host molecules in addition to fibronectin, including laminin, human contact phase proteins, and MHC class I (174–176). Most bacteria optimally express curli at temperatures of 30°C or lower, consistent with a central role in biofilm formation and colonization of environmental surfaces. However, many clinical *E. coli* strains, including UPEC isolates, express curli at host temperature (37°C) (177, 178). In addition to conferring adhesive and aggregative properties to bacteria, curli expression is sensed by the host and modulates host immune responses (177, 179).

Curli share many properties with eukaryotic amyloid fibers. Amyloid fibers are typically associated with human neurodegenerative illnesses such as Alzheimer's, Parkinson's, and prion-mediated diseases (180, 181). In contrast to these diseases, which are thought to be due to uncontrolled protein folding, curli belong to a growing class of fibers termed 'functional amyloid', whose expression is controlled and directed for the benefit of the expressing cell (182, 183). Curli assemble as thin, tangled fibers that are extraordinarily stable and impart important physiological properties to bacteria, some of which play significant roles during host-pathogen interactions. The pathway for assembly of curli on the bacterial surface is distinct from the CU pathway and other pilus assembly systems, and instead utilizes an extracellular nucleation-precipitation mechanism, in which curli subunit proteins are first secreted to the cell surface before being incorporated into the growing fiber.

Curli structure

Curli form densely aggregated masses on the bacterial surface (Fig. 4B). Individual curli fibers measure 3 nm to 4 nm in diameter and are of varying lengths (173, 184, 185). Similar to eukaryotic amyloid, curli fibers are non-branching, rich in β-sheet structure, and highly resistant to the action of proteases and denaturants (182, 184, 186). Curli and other amyloid fibers also share the

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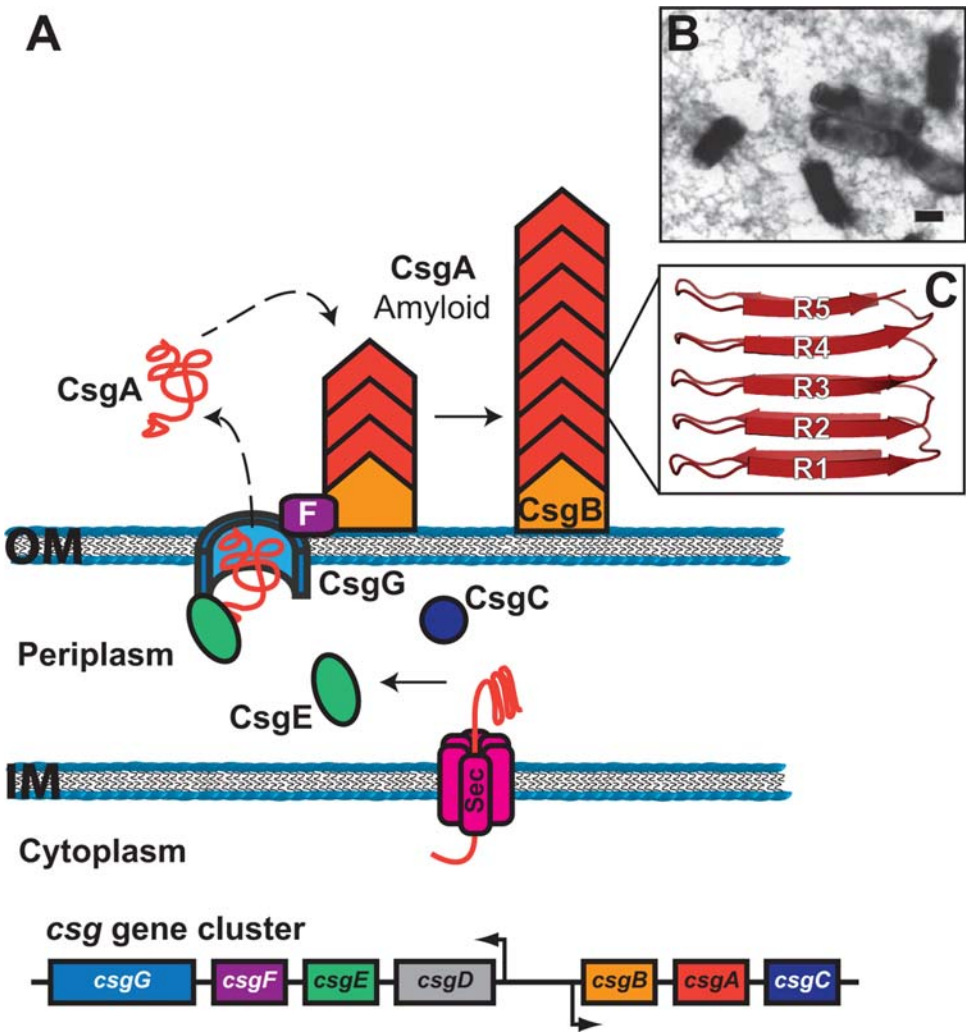

FIGURE 4 **(A)** Model for curli biogenesis by the extracellular nucleation/precipitation pathway. The *csg* gene cluster coding for curli biogenesis is shown at the bottom. The curli subunit proteins enter the periplasm via the Sec system and are secreted to the bacterial surface via the CsgG outer membrane channel. CsgE may act as a chaperone for the curli subunits in the periplasm, whereas CsgF assists assembly of CsgB on the cell surface. Polymerization of CsgA occurs on the cell surface and is nucleated by interaction with CsgB. **(B)** Electron micrograph of *E. coli* expressing curli. Scale bar equals 1 μm; reprinted from reference (205) with permission of the publisher. **(C)** Structure of a CsgA subunit, with the R1-R5 repeats indicated. doi:10.1128/microbiolspec.UTI-0018-2013.f4

property of binding to specific dyes such as Congo red and thioflavin T (187).

Curli fibers expressed by *E. coli* are composed of repeating copies of the major subunit protein, CsgA (Fig. 4A). Each CsgA molecule contains five conserved repeating units (R1 through R5), which are predicted to form two parallel, stacked β-sheets containing five β-strands each (Fig. 4C) (16, 188). Curli fibers also contain a minor, nucleating subunit; in *E. coli* this is CsgB. CsgB shares 30% sequence identity with CsgA, both proteins are of identical predicted size, and both are built from similar repeat motifs (189). The R1 and R5 repeat units, which flank the N and C termini of CsgA, mediate intersubunit contacts and are important for CsgB-mediated nucleation of the CsgA fiber as well as for CsgA-CsgA polymerization (190). In contrast, the R2-to-R4 internal repeats govern the kinetics of fiber polymerization, slowing the rate of polymerization to limit toxicity during curli production (190). Structural analysis indicates that the individual CsgA subunits

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in the curli fiber stack on top of each other, forming an extended β-helix-like structure (185). Therefore, the final fiber consists of an expanse of β-sheets oriented parallel to the fiber axis, but with the individual β-strands oriented perpendicular to the fiber axis. This cross β-strand structure is a hallmark of amyloid fibers (181, 185).

The exact binding sites present on curli fibers and the mechanism by which curli bind to a wide range of receptors is unknown. A study examining synthetic peptides corresponding to overlapping regions of the CsgA sequence identified N- and C-terminal regions of 24 and 26 residues, respectively, that recapitulated binding to several different human proteins (176). In addition, CsgB may have a direct role in adhesion separate from its role in nucleating polymerization of CsgA. This is suggested by studies in *S. enterica*, in which a deletion of *csgB*, but not *csgA*, decreased adherence to alfalfa sprouts (191).

Curli assembly on the bacterial surface

The genes required for curli biogenesis in *E. coli* are encoded by the divergently transcribed *csgBAC* and *csgDEFG* operons (Fig. 4A) (192). The *csgBAC* operon encodes the major structural subunit CsgA and the nucleator protein CsgB (189, 192). CsgC is a periplasmic protein with structural similarity to oxido-reductases (193). The function of CsgC is not understood, but it may be important for proper function of the CsgG outer membrane channel (193). Expression of the *csgBAC* operon is dependent on the positive regulator CsgD, which is part of the *csgDEFG* operon (192, 194).

Extracellular nucleation-precipitation

Assembly of curli on the bacterial surface occurs by an extracellular nucleation-precipitation pathway (16). In the absence of the CsgB nucleator protein, curli are not assembled; instead, CsgA is released from the bacteria in an unpolymerized, soluble form (184, 189). This released CsgA can be assembled into curli fibers on recipient cells expressing only CsgB (189). This process, termed interbacterial complementation, demonstrates that curli assembly may take place entirely on the bacterial surface, and that assembly likely involves a conformational change in CsgA that is triggered by CsgB. Thus, CsgA is secreted outside the bacteria as a soluble, unstructured monomer, which is then nucleated into a fiber on the cell surface by interaction with CsgB or with the structurally altered CsgA in the growing curli fiber (Fig. 4A) (182, 195).

Curli assembly machinery

The secretion and polymerization of CsgA is dependent on the CsgE, CsgF and CsgG proteins, the functions of which are not fully understood (192). CsgG is an outer membrane lipoprotein that is thought to form the channel for secretion of CsgA and CsgB to the cell surface (196). In the absence of CsgG, curli fibers are not assembled and the CsgA and CsgB subunits become unstable (184). Consistent with a channel protein, CsgG forms oligomeric, ring-shaped complexes and overexpression of CsgG correlates with increased pore-formation in the outer membrane (196). Structural analysis of CsgG predicts that it belongs to the recently characterized class of transporters that assemble in the outer membrane as α-helical rather than β-barrel channels (193). The CsgG-mediated secretion of CsgA is dependent on the N-terminal 22 amino acids of the mature CsgA protein. These residues are not predicted to be an integral part of the curli fiber, suggesting that they act as a secretion signal (196, 197).

CsgE is a periplasmic protein and its expression is important for stability of the CsgA and CsgB subunits (184). Consistent with a role in proper folding of the curli subunits, *csgE* mutant bacteria do not act as donors or acceptors for interbacterial complementation and the few curli fibers produced by *csgE* mutants are morphologically distinct from curli expressed by wild-type cells (184). CsgE physically interacts with CsgG at the outer membrane, and CsgE may chaperone periplasmic CsgA subunits to the CsgG secretion channel by interacting with the N-terminal CsgA signal sequence (198). CsgE may also function to prevent premature fiber assembly in the periplasm (198). The CsgF protein also interacts with CsgG at the outer membrane; however, CsgF localizes to the cell surface rather than the periplasm (199). *csgF* mutants have a distinct phenotype, producing reduced levels of curli fibers and secreting soluble, unpolymerized CsgA (184, 199). Similar to *csgB* mutants, *csgF* mutants act as donors but not acceptors for interbacterial complementation. In agreement with this behavior, CsgF influences the folding of CsgB and localization of CsgB to the bacterial surface, suggesting that CsgF functions as an extracellular chaperone for CsgB (199).

Functions of curli in UPEC

Curli are multifunctional surface fibers, conferring adhesion to specific host molecules, promoting bacterial community behaviors such as aggregation and biofilm formation, and modulating interactions with the host immune system (16, 170–172, 178, 200). Curli bind to

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the extracellular matrix proteins fibronectin and laminin (173, 174). Curli also bind to human contact phase proteins including H-kininogen, fibrinogen, and factor XII (175, 176). By binding to the contact phase proteins, curliated bacteria slow clotting, which could facilitate bacterial dissemination throughout the host (177, 201). In addition, curli interact directly with molecules of the immune system. MHC class I molecules, which present antigens to T cells, bind to curli and curliated bacteria adhere better to tissue culture cells that over-produce MHC class I (202).

UPEC and other *E. coli* isolates produce curli at the host temperature of 37°C, including UPEC strains freshly isolated from the urine of infected patients (178, 201). This supports a functional role for curli in colonization of the urinary tract. Curli expression enhances adhesion to urothelial cells in cell culture, and the ability to express curli correlates with increased colonization of the urinary tract during early stages of the murine infection model (178, 203, 204). Curli-mediated binding to host molecules may also facilitate uptake inside host cells (16, 172, 173). Expression of curli genes promoted invasion of human epithelial cells by a non-pathogenic K12 strain of *E. coli*, and invasion was inhibited by addition of peptides that blocked curli formation (172, 205).

In addition to binding to specific host molecules, a major functional role of curli in UPEC is likely promoting bacterial aggregation and biofilm formation. Indeed, curli were shown to contribute to biofilm formation by UPEC distinct from the action of type 1 pili (203). Finally, curli expression by UPEC appears to be an important modulator of host immune responses during infection. Curli fibers are recognized by host cells as a PAMP (pathogen-associated molecular pattern) (200). Curli recognition is mediated by TLR2, resulting in the activation of pro-inflammatory molecules such as IL-6, IL-8, and TNF-α (177, 200). A recent study demonstrated multiple functions for curli during infection of the murine urinary tract: facilitating colonization, protecting the bacteria from the action of host antimicrobial peptides, and provoking an increased pro-inflammatory response (178). Taken together, these results demonstrate that curli play important and varied roles during both initial colonization and subsequent stages of the infectious process.

AUTOTRANSPORTERS AND OTHER NON-PILUS ADHESINS

In addition to assembling adhesins in the form of extended pili or curli fibers, Gram-negative uropathogens also display adhesins directly on their cell surface. The majority of these non-pilus adhesins are assembled on the outer membrane by the autotransporter (type V) secretion pathway (206, 207). Autotransporters are a widespread family of secreted proteins with activities ranging from proteases and toxins to adhesins and invasins. The term autotransporter was first used by Meyer and colleagues to describe the IgA1 protease of *Neisseria meningitidis*, and refers to the idea that a single polypeptide encodes both functional and secretion activities (208). The range of autotransporter functions is reflected by additional well-studied autotransporters, including the NalP protease of *N. meningitidis*, the VacA cytotoxin of *Helicobacter pylori*, and the Pertactin and AIDA-I adhesins of *Bordetella pertussis* and *E. coli*, respectively (209–213). Autotransporters are characterized by the presence of a conserved C-terminal translocator or β-domain that inserts into the outer membrane and directs the secretion of an N-terminal passenger or α-domain, which carries the functional activity, to the cell surface (206, 207). Following secretion, the passenger domain may remain tethered to the outer membrane by the translocator domain or may undergo proteolytic cleavage to be released into the extracellular environment (Fig. 5) (207, 209). In some cases, such as with AIDA-I and related autotransporters, the passenger domain remains associated with the cell surface even after proteolytic cleavage, through noncovalent interactions with the translocator domain (214).

The contributions of autotransporters to bacterial pathogenesis in the urinary tract are still being defined, and their identification has largely proceeded from genomics studies and efforts to characterize UPEC-specific virulence factors (Table 1). The relative paucity of information on surface-located adhesins compared to pili is due to the expression of pili or other extended surface structures may obscure or sterically hinder the functions of proteins present at the bacterial surface (215, 216). Thus, autotransporter adhesins are likely to be important under conditions in which pili expression is turned off. Eleven autotransporters have been identified in the genome of UPEC strain CFT073, a prototypical pyelonephritis isolate (153, 217, 218). Seven of these belong to the AIDA-I family of autotransporter adhesins, and at least four of the UPEC autotransporters function in adhesion to host cells and contribute to fitness in the urinary tract, as discussed below. At least one of the other UPEC autotransporters, Sat, is not an adhesin but is an important protease and toxin of UPEC (219). In addition to UPEC,

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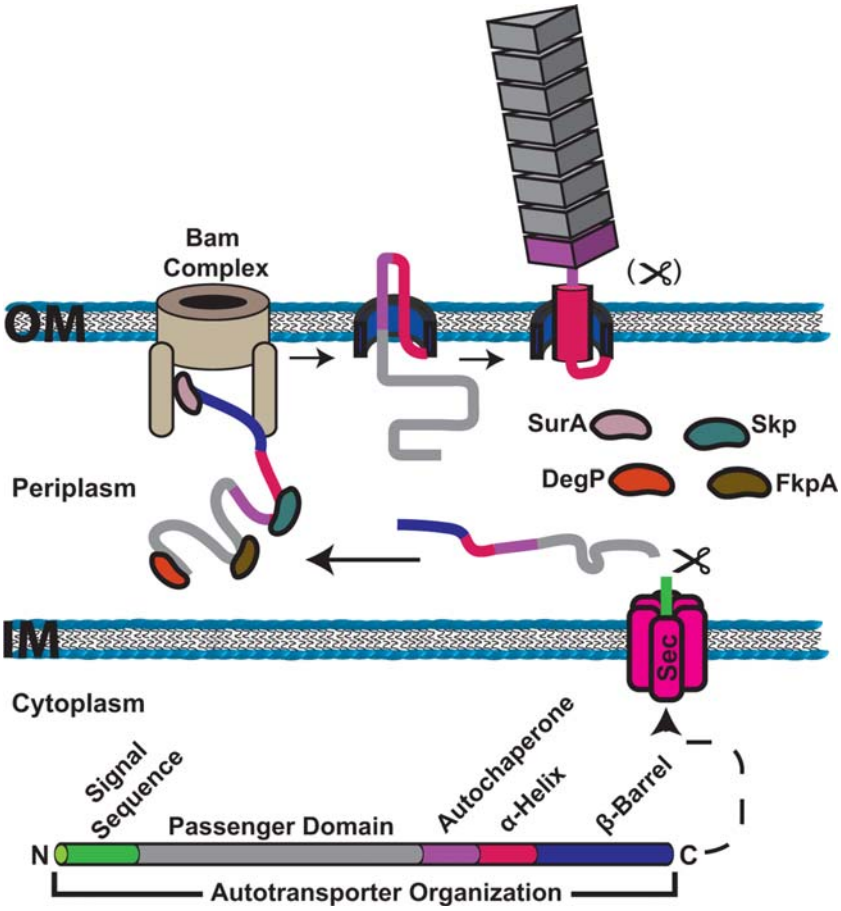

FIGURE 5 Model for autotransporter secretion and assembly on the bacterial surface. The domain organization of an autotransporter protein is shown at the bottom. Autotransporter polypeptides have an N-terminal signal sequence for translocation to the periplasm via the Sec system. The protein is maintained in an extended, largely unfolded state during transit across the periplasm, assisted by periplasmic folding factors (SurA, Skp, DegP and FkpA). The C-terminal translocator domain inserts into the outer membrane as a β-barrel channel, with the assistance of the Bam complex. The Bam complex may also assist in secretion of the passenger domain to the cell surface. In the hairpin model of secretion, the C-terminal region of the passenger domain forms a hairpin structure in the translocator channel, exposing part of the passenger to the cell surface. Folding initiates at the autochaperone region, which then nucleates folding and secretion of the rest of the passenger domain. Following secretion, the linker region adopts an α-helical structure to plug the translocator domain channel. The passenger domain may remain linked to the translocator domain or may be proteolytically cleaved. doi:10.1128/microbiolspec.UTI-0018-2013.f5

autotransporter adhesins that contribute to colonization of the urinary tract have also been identified in *P. mirabilis* (220).

This section will provide an overview of the structure and assembly of autotransporters, and will describe the functions of autotransporter adhesins that have been characterized in UPEC. We will also describe two additional non-pilus adhesins expressed by UPEC that are assembled by distinct mechanisms.

Autotransporter Structure

The translocator domain

Autotransporters contain a C-terminal translocator domain and N-terminal passenger domain. The translocator domain is the most conserved feature of autotransporters, whereas passenger domains exhibit a high level of sequence variation (221). Translocator domains belonging to the classical (type Va) autotransporter family are typically 250 to 300 residues in length and insert into the outer membrane to form a β-barrel channel. Crystal structures for several translocator domains have been solved, revealing a typical outer membrane β-barrel structure comprising 12 antiparallel transmembrane β-strands, enclosing a channel of approximately 10 to 13 angstrom (Å) diameter (Fig. 6) (210, 222, 223). An α-helical linker region important for secretion of the passenger domain precedes the β-barrel, and the helix and barrel together have been termed the translocation unit (224–226). In the autotransporter structures, the α-helical linker occupies the lumen of the β-barrel channel (Fig. 6) (210, 222, 223). The N terminus of the α-helix is oriented toward the bacterial surface, suggesting that translocation of the passenger domain would occur through the β-barrel channel. However, as discussed below, there is debate about the exact mechanism of passenger domain secretion.

Some autotransporters have smaller translocation domains, consisting of only approximately 70 to 80 amino acids. These proteins trimerize to form a single 12-stranded β-barrel, with each monomer contributing 4 strands (Fig. 6) (227–229). The Hia and YadA

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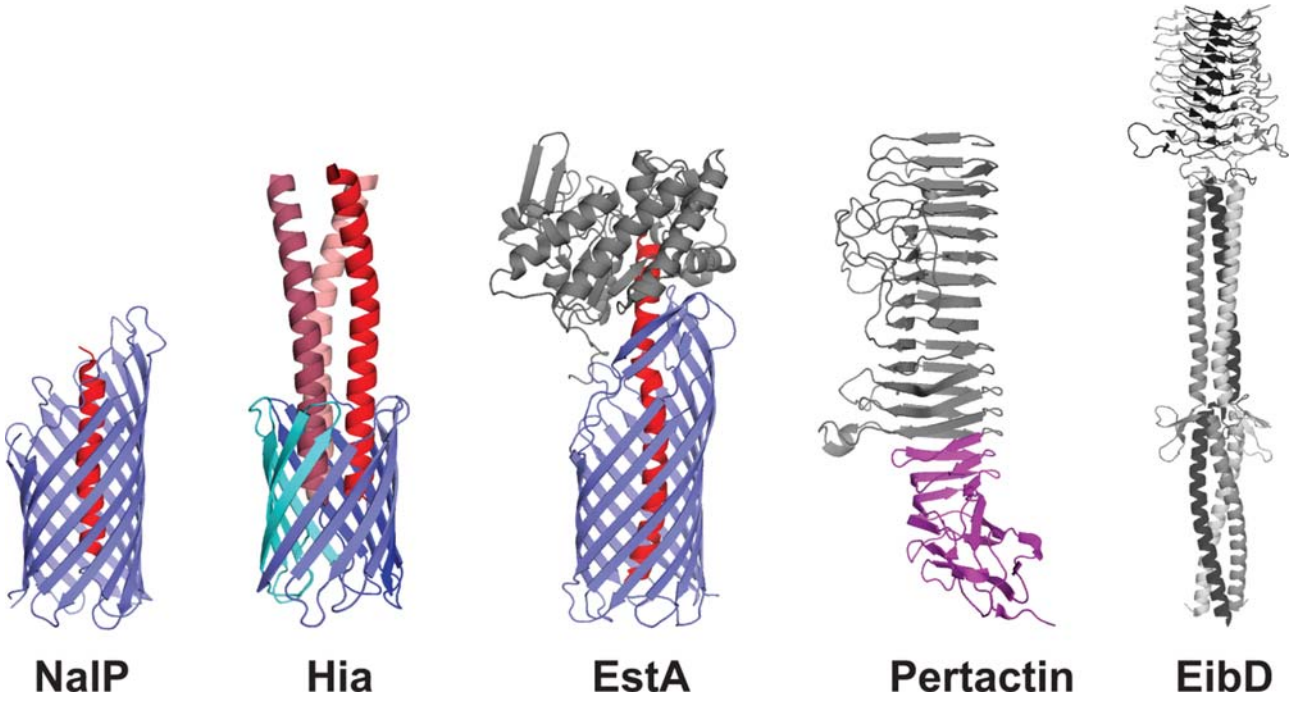

FIGURE 6 Crystal structures of representative autotransporter proteins. Translocator domains from the monomeric NalP and trimeric Hia autotransporters are shown (PDB IDs: 1UYN and 2GR7, respectively), with the β-barrel channels in blue and the α-helical linker regions in red. Passenger domains from the monomeric Pertactin and trimeric EibD autotransporters are shown (PDB IDs: 1DAB and 2XQH, respectively), with the approximate location of the Pertactin autochaperone region indicated in purple. The complete structure of the EstA autotransporter is shown (PDB ID: 3KVN), with the translocator domain in blue, the α-helical linker in red, and the globular passenger domain in gray. doi:10.1128/microbiolspec.UTI-0018-2013.f6

adhesins of *H. influenza*e and *Y. pestis* are prototypical members of this subfamily (type Vc), termed trimeric autotransporter adhesins (206, 230, 231). The β-barrel formed by the Hia translocator domain has a central pore of 18 Å diameter, which is sufficient to accommodate passage of the three α-helical linker segments that connect to the extracellular passenger domains (Fig. 6) (229). Of the 11 autotransporters identified in UPEC, only UpaG belongs to the trimeric subfamily (232).

The passenger domain

The N-terminal passenger domains of autotransporters are structurally and functionally diverse, but share important conserved features. Most passenger domains are large in size and contain repetitive sequence motifs that assemble into repetitive structural elements (206, 207). The sequence motifs typically form β-sheets that are arranged into an extended β-helix, with each rung of the helix comprising three β-strands in a triangular arrangement (Fig. 6) (212, 233–235). In different autotransporters, this β-helix core structure may be modified with interspersed extended loops, globular domains, or other elements, which contain specific functions such as receptor binding sites or protease activity. However, not all passenger domains have a β-helix architecture. For example, the complete structure of the EstA autotransporter from *Pseudomonas aeruginosa* revealed a typical 12-stranded β-barrel translocator domain, but a globular passenger domain primarily composed of α-helices and loop sequences (Fig. 6) (222).

Passenger domains from the trimeric autotransporter subfamily exhibit a distinct architecture compared to the classical autotransporters, but also assemble into extended structures built from repetitive sequence elements. Members of this family typically function as adhesins and the passenger domains remain attached to the outer membrane translocator rather than undergoing proteolysis. The passenger domains trimerize, matching the trimeric structure of the translocator

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domain, and form extended rod-shaped structures. A common architecture is shared by the trimeric passenger domains, comprising a globular N-terminal head region with extensive β-sheet structure, followed by an extended coiled-coil stalk region that connects to the translocator anchor domain in the outer membrane (Fig. 6) (230, 231, 236–238). Some trimeric autotransporters have more complex architectures, with modular arrangements of interspersed head, neck, and stalk regions (236, 237). The head region typically contains the receptor binding site (Fig. 6), which may be present in each monomer and thus displayed in triplicate around the surface of the trimer, such as for the Hia adhesin (230). The stalk region may also have binding activity for host molecules or mediate bacterial-bacterial interactions (238, 239).

The Autotransporter Secretion Pathway

Transit across the periplasm and insertion into the outer membrane

Nascent autotransporter polypeptides are synthesized with an N-terminal signal peptide that is cleaved following translocation of the protein from the cytoplasm to the periplasm via the Sec general secretory pathway (78). The passenger domain must be kept in a largely unfolded state to remain competent for secretion. Resident periplasmic chaperones and folding factors (DegP, FkpA, SurA, and Skp) interact with the extended autotransporter polypeptide in the periplasm (Fig. 5) to maintain the passenger domain in a secretion-competent state and prevent non-productive interactions, as well as assist in proper folding and insertion of the translocator domain in the outer membrane (206, 207, 240, 241). Some autotransporters have an extended N-terminal Sec signal sequence that slows the rate of translocation across the cytoplasmic membrane through the Sec system (207, 242, 243). This slowing of translocation may facilitate proper transit of the autotransporter polypeptide across the periplasm.

Secretion of the N-terminal passenger domain to the cell surface requires the C-terminal translocator domain, which inserts into the outer membrane to form a β-barrel channel. Recent studies have defined a set of proteins in the bacterial outer membrane, termed the β-barrel assembly machine (Bam) complex, which is responsible for proper insertion of most β-barrel proteins into the outer membrane (244). In keeping with this, correct assembly of the translocator domain in the outer membrane and its proper functioning in autotransporter secretion requires the Bam complex (245–247).

Secretion of the passenger domain to the cell surface

The exact mechanism by which the passenger domain is secreted from the periplasm to the bacterial surface remains a topic of active investigation. Two main models have been proposed for this process: a 'classical' hairpin model and a newer model that invokes a central role for the Bam complex (206, 207).

In the hairpin model, secretion of the passenger domain occurs through the lumen of the β-barrel channel formed by the translocator domain. Secretion of the passenger domain initiates when a C-terminal region of the passenger domain, likely including the α-helical linker region, forms a temporary hairpin structure within the pore (Fig. 5). Formation of the hairpin exposes part of the C terminus of the passenger domain to the cell surface, where it may begin folding. Folding of the passenger domain would then proceed vectorially from the C to the N terminus, progressively pulling the polypeptide through the channel (248, 249). The hairpin model requires the presence of two strands of the passenger domain polypeptide within the lumen of the translocator domain pore. Given the narrow dimensions of the pore, these strands would need to be in a largely unfolded and extended conformation, which is consistent with studies showing a general lack of tolerance for structured elements in passenger domains (241, 250, 251).

Autotransporter secretion across outer membrane does not require the input of energy from the cytoplasmic membrane, and folding of the passenger domain at the cell surface likely provides the energy to drive secretion through the translocator channel (248, 249). Folding at the cell surface could also act as a ratchet to prevent diffusion of the passenger back into the periplasm. Most passenger domains contain a conserved junction sequence adjacent to the α-helical linker, termed the autochaperone domain. This region, which is critical for the folding of β-helical passenger domains, would be exposed to the cell surface upon formation of the hairpin loop and may act as an intramolecular chaperone to nucleate folding of the rest of the passenger domain on the cell surface (Figs. 5 and 6) (252–254). Progressive folding of rest of the passenger domain would then occur through a self-templating mechanism, driven by the repeated β-helix structure (255). Once secretion of the passenger domain is complete, the linker region would assume its final α-helical conformation to plug the pore, as observed in the autotransporter crystal structures (Figs. 5 and 6) (210, 222, 223).

The classical hairpin model proposes that the β-barrel channel formed by the translocator domain is sufficient

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for passenger secretion and that no other accessory factors are required (which is the basis for the term autotransporter). However, conflicts with this model, particularly in studies showing tolerance for secretion of some folded elements and the presence of post-translationally modified passenger domains (256–258), have led to revised models that invoke a central role for the Bam complex (210, 245, 259). In these models, the β-barrel translocator domain serves to target the autotransporter polypeptide to the Bam complex. Interaction with the Bam complex then allows folding and insertion of the translocator domain in the outer membrane, coupled with secretion of the passenger domain to the cell surface. Rather occurring through the translocator domain channel, secretion of the passenger domain would occur, at least in part, through the BamA channel or through some interface between the Bam complex and the translocator domain (207, 210). As the BamA channel is unlikely to be able to gate laterally to allow release of the passenger domain, secretion of the passenger domain would need to occur in concert with insertion of the translocator β-barrel into the outer membrane, and is likely to involve some aspects of the hairpin model (206, 207, 245, 259, 260). Thus, the Bam complex may facilitate coupled formation of the hairpin structure and insertion of the translocator domain into the outer membrane (Fig. 5), and possibly assist in secretion of structured regions. This would then establish an initiating point from which secretion of the remainder of the passenger domain would proceed through the translocator channel as proposed in the classical hairpin model.

Functions of Autotransporter Adhesins in UPEC

Ag43

Antigen 43 (Ag43) is an autotransporter adhesin encoded by the *flu* gene (also termed *agn43*). Ag43 functions in adhesion to host cells and self-associates to promote bacterial aggregation (autoaggregation), leading to flocculation in static liquid cultures and biofilm formation on surfaces (261–264). Ag43 is present in approximately 80% of UPEC strains, and many strains encode more than one copy (265). UPEC strain CFT073 expresses two Ag43 variants, Ag43a and Ag43b. The Ag43a variant appears to be the functionally relevant form in UPEC, promoting high levels of aggregation, biofilm formation, and colonization of the urinary tract (265). Expression of Ag43 is phase variable and opposite from expression of type 1 pili; expression of the longer pilus fibers on the bacterial surface sterically blocks adhesion mediated by the shorter Ag43 molecules (216, 266).

Ag43 belongs to the AIDA family of autotransporters (267). Similar to AIDA-I, the passenger domain of Ag43 is proteolytically processed following transport to the cell surface, but remains associated with the translocator domain via non-covalent interactions. Also similar to AIDA-I, the Ag43 passenger domain is glycosylated in some *E. coli* strains, including UPEC isolates (258). The importance of glycosylation for Ag43 function remains to be determined, as different studies have found variable effects of glycosylation on autoaggregation, biofilm formation, and adhesion to host cells (258, 263). Ag43 promotes adhesion to various cell lines, including human kidney cells, and binds to the extracellular matrix components collagen and laminin (263). The Ag43 passenger domain contains multiple repeats of approximately 19 residues each and folds with an extended, L-shaped β-helical structure (268). The region of Ag43 responsible for autoaggregation is located in the first N-terminal third of the mature passenger domain (261). Recent structural analysis of the Ag43 passenger domain suggests that self-association is mediated by a "Velcro-like" mechanism (268).

Several lines of evidence point to a role for Ag43 in UTIs. Anderson and colleagues found that Ag43 is expressed during bladder infection by UPEC strain UTI89 (143). Specifically, Ag43 was present on the surface of bacteria engaged in formation of intracellular biofilm-like communities following invasion of bladder epithelial cells. Consistent with this observation, the Ag43a variant was found to promote long-term persistence of UPEC strain CFT073 in the murine UTI model; a *fluA* but not *fluB* mutant of CFT073 is present at lower numbers in the bladder compared to the parental strain at day 5 post infection (265). In addition, expression of Ag43 may promote formation of linked bacterial chains that are formed by asymptomatic bacteriuria *E. coli* isolates when grown in human urine (269). The autoaggregation properties of Ag43 are likely to enhance bacterial colonization of the urinary tract as well as formation of intracellular and extracellular biofilms.

UpaB

UpaB is an autotransporter adhesin identified in UPEC strain CFT073, and is widely distributed among both uropathogenic and non-uropathogenic *E. coli* strains (270). UpaB belongs to the AIDA-I family of autotransporters and contains a predicted pertactin-like passenger domain (270). UpaB confers binding to extracellular

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matrix proteins, including fibronectin, fibrinogen, and laminin. A *upaB* deletion mutant of CFT073 is outcompeted by the wild-type strain for colonization of the bladder, and the mutant strain is specifically defective for an early stage of bladder colonization (270). However, a direct role for UpaB in adhesion to the urinary tract has not been demonstrated. UPEC encode an additional autotransporter related to UpaB, termed UpaC; however, UpaC is not expressed by CFT073 and a UpaC mutant had no phenotype in the murine UTI model (270).

UpaG

UpaG is a trimeric autotransporter adhesin prevalent among extraintestinal pathogenic *E. coli* (ExPEC) strains belonging to the B2 and D phylogenetic groups, including UPEC strain CFT073 (232). The structure of UpaG has been reconstructed from crystal structures of fragments of the homologous *Salmonella enterica* protein SadA (237). UpaG assembles as an extended coiled-coil fiber, ~115 nm in length, containing four YadA-like head repeats and adaptor neck regions typical of trimeric autotransporters (237). Expression of UpaG in CFT073 promotes adhesion to the T24 human bladder epithelial cell line, with specificity for fibronectin and laminin, and promotes autoaggregation and biofilm formation (232). UpaG was identified as a potential protective antigen of ExPEC, suggesting that it is expressed during infection (271). However, native expression of UpaG was not detected in CFT073 grown under in vitro conditions, and no role was found for UpaG in colonization of either the bladder or kidneys using the murine infection model (232).

UpaH

The UpaH autotransporter adhesin is expressed by the CFT073 UPEC strain, where it provides a competitive advantage for colonization of the bladder and contributes to biofilm formation (217). UpaH is a large-sized (~280 kDa) member of the AIDA-I family of autotransporters (272). UpaH binds to the extracellular matrix proteins collagen V, fibronectin, and laminin (272). However, a direct role for UpaH in adhesion to the urinary tract has not been demonstrated. The *upaH* gene is present in the chromosomes of many UPEC isolates, as well as in non-uropathogenic *E. coli* strains (217). Bioinformatics analysis predicts a typical 12-stranded β-barrel translocator domain and a large passenger domain with 50 imperfect sequence repeats predicted to encode an extended β-helix structure (217). Sequence variation is present in the UpaH passenger domain from different *E. coli* isolates (272). These variations were found to impact function in biofilm formation but not binding to extracellular matrix proteins.

FdeC

FdeC was identified in a screen for ExPEC vaccine antigens that provided protection in a murine sepsis model (273). The *fdeC* gene is widely distributed among ExPEC and also intestinal *E. coli* strains (274). FdeC shares a low level of sequence homology with the invasin and intimin proteins of *Yersinia pseudotuberculosis* and enteropathogenic *E. coli*, respectively, which function in adhesion to host cells (275, 276). Similar to these proteins, FdeC is anchored in the outer membrane via a presumed N-terminal β-barrel domain, with the extracellular portion of the protein forming an elongated structure comprising nine repeated Ig-like domains (274). A model was recently proposed that proteins such as intimin and invasin form a new subfamily of autotransporters (type Ve) (206, 277). In this model, the proteins are secreted in an analogous mechanism to autotransporters, but with a reverse topology (i.e., the outer membrane translocator domain is located at the N terminus instead of the C terminus as for typical autotransporters). In contrast to intimin and invasin, no obvious lectin domain is present in FdeC (274). Recombinant FdeC binds to human urothelial cell lines, as well as other types of epithelial cells, with specificity for collagen (274). FdeC is expressed during interactions with host cells and during infection of the urinary tract, and a *fdeC* mutant of UPEC strain 536 was defective for colonization of the bladder and kidneys during coinfection with the wild-type strain (274).

Other Outer Membrane-Associated Adhesins of UPEC

At least two additional non-pilus adhesins that contribute to pathogenesis in the urinary tract have been identified in UPEC. These adhesins are assembled on the bacterial outer membrane by mechanisms that are distinct from the autotransporter pathway. The Iha adhesin is secreted by the type I secretion pathway and TosA, a multifunctional siderophore receptor and adhesin, is an integral outer membrane protein.

Iha

The IrgA homologue adhesin (Iha) protein, encoded by the *iha* gene, is prevalent among UPEC strains and has the novel phenotype of functioning in both adhesion and iron uptake (278, 279). Iha was originally identified in the diarrheagenic *E. coli* strain O157:H7 as an adhesin

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with homology to the *Vibrio cholerae* iron-regulated virulence factor IrgA (280). Iha is an outer membrane protein with homology to β-barrel siderophore receptor proteins such as FepA (281). *Iha* is a virulence factor of UPEC, as demonstrated by reduced fitness of a CFT073 *iha* deletion mutant for colonization of both bladder and kidneys in the murine UTI model (279). Similarly, an *iha* mutant of the UPEC clonal group A outbreak strain UCB34 (282) was attenuated for infection of the urinary tract in competition with the parental wild-type strain (278). There is also evidence supporting a role for *iha* in the pathogenesis of UTIs caused by *Proteus mirabilis* (283). *Iha* is expressed in vivo during infection of the murine urinary tract (278). Expression of recombinant Iha promoted adhesion to human epithelial cells, including the T24 bladder cell line, whereas overexpression of other siderophore receptors did not promote adhesion (278, 279). In addition to its function as an adhesin, Iha functions in iron uptake as an iron-regulated catecholate siderophore receptor (278). Given the likely topology of Iha as in integral outer membrane β-barrel protein, adhesive activity is presumably conferred by surface-exposed loops of the protein. Whether the adhesin function, iron uptake, or both are important for pathogenesis remains to be determined.

TosA

TosA belongs to the repeats-in-toxin (RTX) family of secreted bacterial proteins that have diverse functions, including acting as toxins, proteases, and adhesins (284). RTX proteins share the characteristic features of repetitive glycine- and aspartate-rich sequences, located in the C-terminal region of the protein, and use of the type I secretion system for export out of bacteria. Type I secretion systems function in the secretion of a variety of toxins and other virulence factors directly from the cytoplasm to the extracellular milieu in a single energized step (285, 286). The type I system comprises three components: an outer membrane channel-forming protein (TolC in *E. coli*), a periplasmic adaptor or membrane fusion protein, and an inner-membrane pump that typically belongs to the ATP-binding cassette family. In contrast to most RTX proteins, TosA remains associated with the bacterial surface following secretion, rather than being released into the external environment (287). The *tosA* gene is present in a pathogenicity island in UPEC strains, particularly those of the B2 phylogenetic group, in an operon together with genes encoding a type I secretion system (287, 288). *tosA* is expressed in vivo during infection of the urinary tract (287) and a CFT073 Δ*tosA* mutant was defective for colonization of both the bladder and kidneys in the mouse UTI model (289). Evidence suggests that *tosA* also enhances fitness during disseminated infections (287). Expression of TosA promotes adherence to both murine and human kidney epithelial cells, but does not appear to be important for colonization of the lower urinary tract (287).

ADHESINS EXPRESSED BY GRAM-POSITIVE UROPATHOGENS

While Gram-negative bacteria are responsible for the majority of UTIs, Gram-positive bacteria are also significant uropathogens. *Staphylococcus saprophyticus* is the second leading cause of community acquired UTIs in sexually active women, accounting for approximately 15% of all incidences (290). *Enterococcus faecalis*, a normal member of the gut flora, is also a causative agent of UTIs, particularly in nosocomial infections (291). The adhesive organelles of Gram-positive bacteria differ significantly from those of Gram-negative bacteria in structure and assembly mechanism, but fall into the same two general classes of pilus or surface-located adhesins. Gram-positive pili, like their Gram-negative counterparts, consist of multiple pilin subunits linked together to form an extended, hair-like fiber (21, 22, 292). The majority of non-pilus adhesins associated with UTIs are multi-domain proteins known as MSCRAMMs (microbial surface components recognizing adhesive matrix molecules), which are anchored directly to the bacterial cell wall (22, 23).

MSCRAMMs

The extracellular matrix is a complex protein network that serves as the major scaffolding component of eukaryotic cells and tissues, and mediates numerous essential cellular processes including morphogenesis and differentiation (293, 294). Gram-positive bacteria utilize components of the extracellular matrix such as collagen, fibronectin, and laminin as binding ligands to promote adherence, colonization, and biofilm formation (295–297). To achieve this, Gram-positive bacteria express the MSCRAMM family of surface-associated adhesins. MSCRAMMs are multi-domain proteins that are covalently linked to the peptidoglycan cell wall, exposing both conserved and non-conserved regions to the extracellular milieu. Different MSCRAMM domains confer specific functionality, including ligand binding, cell wall anchoring, and structural integrity (22). Crystal structures have been solved for domains from several different MSCRAMMs, including SdrG from *Staphylococcus epidermidis*, Ace from *E. faecalis*, and Cna and

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ClfA from *Staphylococcus aureus*, providing a structural context for understanding the assembly and functions of MSCRAMMs (298–305). MSCRAMMs that have been associated with uropathogenic bacteria are Ace, expressed by *E. faecalis*, and UafA, UafB and SdrI, expressed by *S. saprophyticus* (306–309).

Structure of MSCRAMMs

All MSCRAMMs share a common domain organization. The N terminus contains a signal peptide that directs the proteins for translocation across the cytoplasmic membrane by the Sec pathway. The N-terminal signal peptide is followed by a multi-domain central region, where the major functional and structural diversity resides (22). The C terminus contains a conserved cell wall sorting signal (CWSS), which consists of the amino acid sequence LPXTG (X represents any amino acid), followed by a hydrophobic transmembrane domain, followed by a positively charged cytoplasmic tail (Fig. 6A). This CWSS is required for the covalent linkage of MSCRAMMs to the cell wall (310).

The MSCRAMM functional region is typically divided into A and B regions. The N-terminal A region is responsible for ligand binding and specificity; the C-terminal B region can have both binding and structural properties. Variations on this organization occur, including the presence of an R structural region instead of or in addition to a B region (22). The C-terminal structural domains function to project the binding domain away from the bacterial surface. Similar to the CU pilins of Gram-negative bacteria, MSCRAMMs, as well as Gram-positive pilins, have exploited the Ig fold as a common building block (58). Two Ig variants present in both MSCRAMMs and Gram-positive pilins are the DEv-IgG and IgG-rev folds (Figs. 7B and 8) (22). These folds were first observed in the A and B regions of the *S. aureus* Cna and ClfA MSCRAMMs (305, 311). A typical IgG constant domain contains two β-sheets (sheets 1 and 2) of four and three β-strands, arranged into a barrel configuration. DEv-IgG folds contain the same overall structure, but with the addition of at least two β-strands between strands D and E of sheet 1. The IgG-rev fold has a typical two sheet, seven-stranded barrel, but the strands are arranged in a reverse order compared to typical Ig folds.

Ace and the collagen hug model

The *E. faecalis* Ace (adhesin to collagen of *E. faecalis*) protein was the first MSCRAMM to be associated with UTIs (295, 297, 306, 312). Much of the structural organization of Ace was delineated based on its homology to Cna (295, 302, 303). The functional portion of Ace is made up of A and B regions. The A region is divided into two DEv-IgG subdomains, N_1 (146 amino acids) and N_2 (135 amino acids). These domains are essential for binding extracellular matrix proteins such as laminin and collagen I and IV (297). Crystal structures have been solved for the Ace N_1 and N_2 subdomains (Fig. 7B) (300, 301). Both subdomains are composed of ten β-strands forming two β-sheets in a sandwich like configuration. A missing G strand in sheet 1 of the N_1 subdomain is complemented by a C-terminal extension of the N_2 subdomain, forming an interface between the two subdomains (Fig. 7B). This interface, in addition to a collagen-binding site on the N_2 subdomain and a linker region connecting the two subdomains, forms a deep, tunnel-like, collagen-binding trench. Together, the N_1 and N_2 subdomains form a dynamic cooperative structure utilizing a "collagen hug" model of ligand binding (301).

Structural and functional studies of both Cna and Ace have led to a mechanistic understanding of ligand binding by the A region. The structures of the N_1 and N_2 subdomains of the A region show a closed form of the molecule, in which the N_1 and N_2 subdomains and the inter-domain linker interact with each other, creating a tunnel to accommodate and secure collagen (Fig. 7B) (302). This closed form of the molecule is unable to initiate binding to collagen, and evidence indicates that the N_1 and N_2 subdomains exist in equilibrium between open and closed conformations (301). In the open conformation, a shallow groove in the N_2 subdomain binds the repeating glycine-proline-hydroxylproline $(GPO)_n$, triple helical peptide of collagen with low affinity. Following binding, the C-terminal extension of the N_2 subdomain orients and inserts into a trench in the N_1 subdomain, complementing a missing N_1 β-strand and allowing the N_1 and N_2 subdomains to come into close proximity of each other, shrinking the hole and "hugging" the collagen molecule in place (301, 302). The N_2 C-terminal extension also acts as a latch, securing the complex. Truncations of the latch cause a decreased affinity for collagen, likely due to the N_1 and N_2 subdomains insufficiently securing the collagen in place (301).

The B region of Ace has not been resolved, but a high degree of sequence homology between this region and the B region of Cna provides insight into its structural organization and function. The Ace B region comprises five repeating domains, $B_{1–5}$ (301). The crystal structure of the Cna B domain indicates that it functions as a structural element rather than in ligand binding (303). The Cna B_1 domain is divided into two IgG-rev fold

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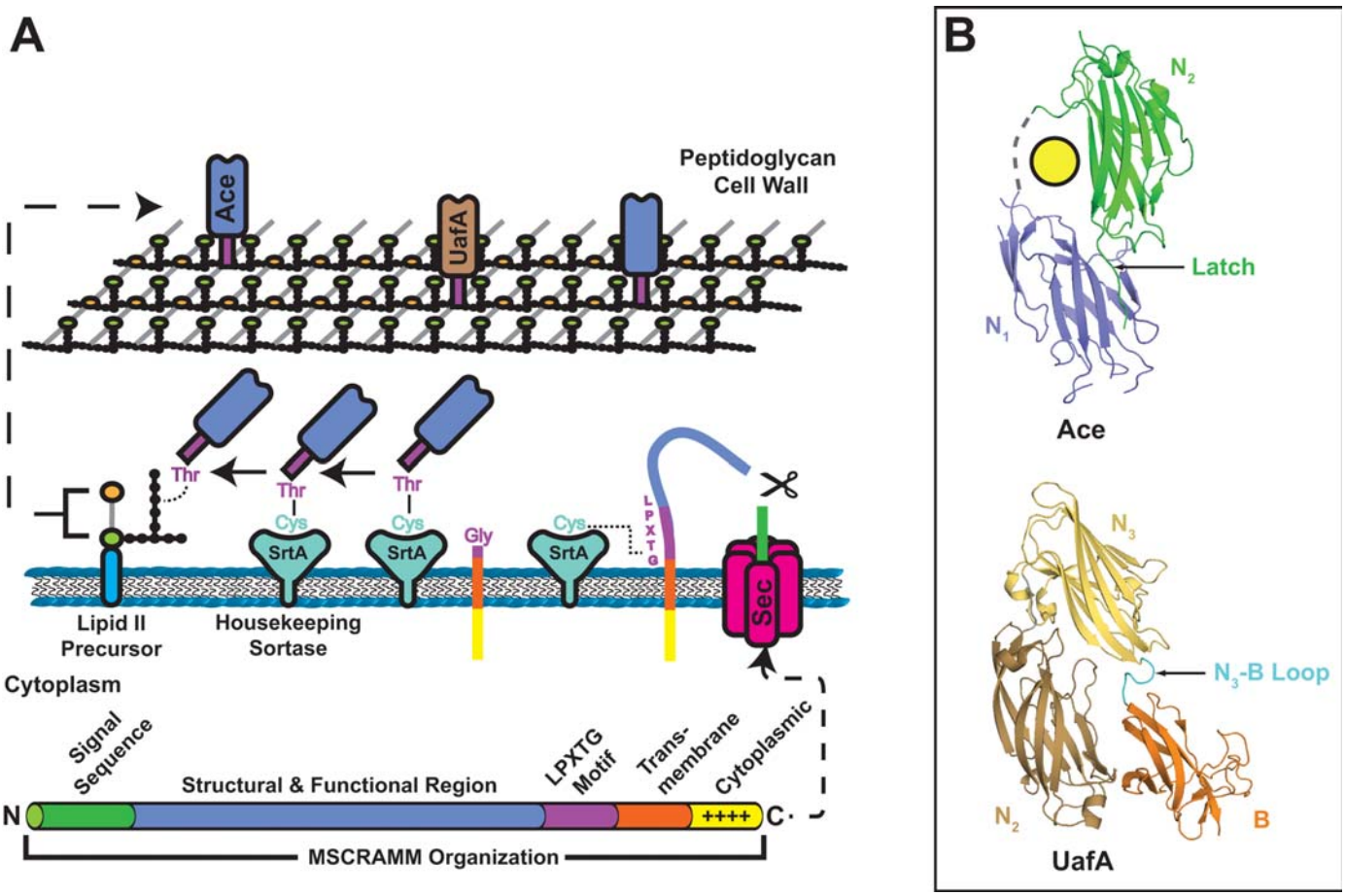

FIGURE 7 **(A)** Model for MSCRAMM secretion and incorporation into the cell wall. The domain organization of a typical MSCRAMM is shown at the bottom. MSCRAMMs have an N-terminal Sec signal sequence for translocation across the cytoplasmic membrane. The protein remains anchored in the cytoplasmic membrane by the CWSS. The positively charged C terminus remains in the cytoplasm, orienting the LPXTG motif to the extracellular side of the membrane. The SrtA sortase cleaves between the Thr and Gly of the MSCRAMM LPXTG motif, forming a covalent thioacyl intermediate. The MSCRAMM is then transferred to a lipid II peptidoglycan precursor and finally integrated into the cell wall at an amino acid cross-bridge. **(B)** Crystal structures of the Ace and UafA MSCRAMMs (PDB IDs: 2Z1P and 3IRP, respectively). The upper structure shows the N_1 and N_2 subdomains of Ace in blue and green, respectively; the yellow circle represents bound collagen. Both domains have DEv-Ig folds. The C terminus of the N_2 subdomain inserts into the N_1 subdomain, forming a latch. The lower structure depicts the N_2, N_3, and B subdomains of UafA. The N_2 and N_3 subdomains adopt DEv-Ig folds and the B subdomain adopts a variant of the IgG-rev fold. The loop connecting the N_3 and B domains (cyan) is thought to insert into the N_2 subdomain upon ligand binding to form a latch. doi:10.1128/microbiolspec.UTI-0018-2013.f7

subdomains, D_1 and D_2. Domains B_{2-5} share the same structure, and the repeating B domains are arranged in an accordion-like fashion, which acts as a stalk supporting the A region and extending it distally from the surface of the cell (303). C terminal to the B region is the characteristic CWSS, which allows anchoring to the peptidoglycan cell wall by the housekeeping sortase enzyme.

UafA and the dock, lock, and latch model

Another MSCRAMM shown to impact bacterial adherence in the urinary tract is UafA (uro-adherence factor A), expressed by *S. saprophyticus* (307). Information obtained from MSCRAMMs such as the *S. aureus* Cna and ClfA, as well as the recent crystal structure of the functional region of UafA, have provided

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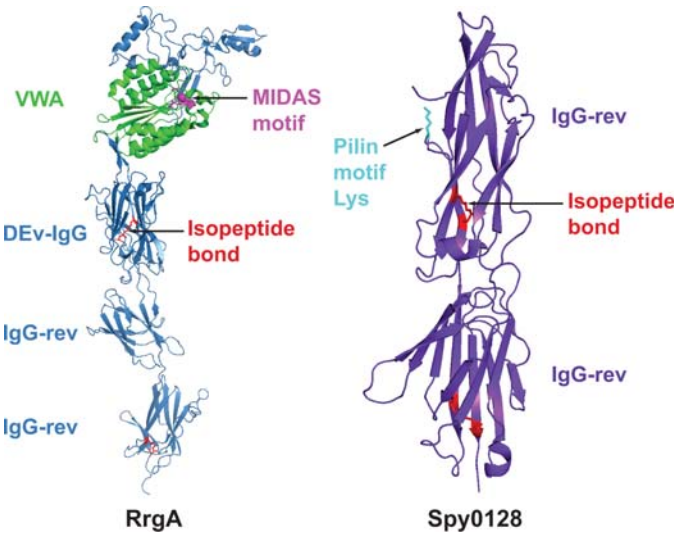

FIGURE 8 Crystal structures of representative tip and major pilins. The RrgA tip pilin of *S. pneumoniae* (PDB ID: 2WW8) is shown on the left, with the fold adopted by each of the four subdomains indicated. The VWA domain is depicted in green, with the residues forming the MIDAS motif and bound magnesium ion shown in purple. The residues involved in intramolecular isopeptide bond formation are shown in red. The Spy0128 major pilin of *S. pyogenes* (PDB ID: 3B2M) is depicted on the right in purple and the two subdomains are labeled as for RrgA. The lysine side chain of Syp0128 thought to be involved in intermolecular isopeptide bond formation is shown in cyan. doi:10.1128/microbiolspec.UTI-0018-2013.f8

insight into the structural organization and function of UafA (Fig. 7B) (313). UafA contains an N-terminal Sec signal sequence, a 72 kDa A region, a 13 kDa B region, a 148 kDa R region, and a C-terminal CWSS. Unlike Ace however, the UafA A region comprises three subdomains, N_{1-3}, and the B region contains a single, nonrepeating domain. The additional R region is rich in serine and glutamine residues and of low complexity (313).

The A region of UafA is required for ligand binding and shares the same variant DEv-IgG folds in its N_2 and N_3 subdomains as seen in Ace, Cna, and ClfA (305, 313). The single B domain does not act as a stalk to extend the A region from the surface, but instead is a required component in ligand binding (313). A short loop links the B domain to the N_3 subdomain, and in the three-dimensional structure the B domain also resides adjacent to the N_2 subdomain (Fig. 7B). The low-complexity R region is thought to form the UafA stalk, supporting both the upstream A and B regions (313).

A "dock, lock, and latch" model has been proposed for UafA, based on similarities with ClfA and the *S. epidermidis* fibrinogen-binding protein SdrG (298, 299, 304). This model is mechanistically related to that of the collagen hug model of Ace and Cna, but has distinct features. The N_3 subdomain is composed of two β-sheets with a total of nine β-strands in the configuration of A, B, D, E, and C, D1, D2, F, G (313). The N_2 subdomain contains two similar β-sheets, but sheet 2 lacks a D β-strand and in its place exists a D loop, which does not appear to hydrogen bond with the adjacent E strand. The dock, lock and latch model proposes that in the apo form of the complex, the ligand is first captured by binding between the N_2 and N_3 subdomains. This docking event then triggers a conformational change to engage the loop connecting the N_3 subdomain and the B domain (Fig. 7B), causing the loop to insert into the pocket between the D and E β-strands of the N_2 domain, completing the missing β-strand and forming the "latch" (298).

The ligand for UafA has not yet been defined. UafA functions in hemagglutination and this activity is dependent on the N_2 and N_3 subdomains, as well as the B domain (313). Hemagglutination studies with proteinase K-treated erythrocytes suggest that the ligand is not a protein, but may instead be a carbohydrate or lipid molecule (313). In addition, the B domain may also have secondary ligand binding properties of its own, independent of the N_2 and N_3 subdomains (313).

Assembly of MSCRAMMs

The majority of surface exposed proteins in Gram-positive bacteria utilize a highly conserved assembly mechanism that facilitates translocation of these proteins across the cytoplasmic membrane and their covalent linkage to the peptidoglycan cell wall. As with most Gram-negative adhesins, MSCRAMMs are synthesized with an N-terminal signal sequence that directs the proteins for translocation across the cytoplasmic membrane via the Sec general secretory pathway. The signal sequence is then cleaved following translocation by the signal peptidase (22, 314). Since Gram-positive bacteria lack an outer membrane, proteins translocated across the cytoplasmic membrane will be lost to the extracellular milieu unless they are anchored to the peptidoglycan cell wall (315). Proteins destined to be anchored to the cell wall, including MSCRAMMs, contain the highly conserved CWSS, comprising the LPXTG motif, a stretch of hydrophobic residues, and a positively charged C-terminal tail. Mutations to this motif abrogate cell wall anchoring (316). Following passage across the cytoplasmic membrane via the Sec pathway, the hydrophobic region of the CWSS forms a transmembrane domain, with the positively charged tail orienting

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the domain such that the C terminus remains in the cytoplasm and the LPXTG motif is exposed on the extracellular surface of the membrane (Fig. 6A). Anchoring of the secreted protein to the call wall is then achieved by processing of the LPXTG motif by membrane-associated proteins known as sortases (317, 318).

Sortases are cysteine transpeptidases responsible for sorting covalently linked cell wall proteins to the bacterial surface, including many virulence factors (319). While different classes of sortases exist, most Gram-positive bacteria express a general housekeeping sortase required for displaying a broad spectrum of proteins with distinct functions (320). Sortase A (SrtA) is the best characterized of these housekeeping sortases (318). SrtA is responsible for processing a newly secreted protein's CWSS by catalyzing a transpeptidation reaction between the LPXTG motif and an amino acid cross-bridge of the peptidoglycan (Fig. 7A). SrtA cleaves the carbonyl carbon between the threonine and glycine residues of the LPXTG motif via nucleophilic attack by the conserved active site cysteine of the sortase (318). This cleavage facilitates the creation of a thioacyl bond between the SrtA cysteine and the threonine of the surface protein, resulting in the two proteins being covalently linked together (Fig. 7A). SrtA then transfers the covalently linked protein to lipid II, a membrane bound peptidoglycan precursor. An amino group of lipid II nucleophilically cleaves the sortase-surface protein thioacyl linkage, forming an isopeptide bond with the surface protein threonine and creating a lipid II-surface protein complex (Fig. 7A). The lipid II-surface protein complex is then modified by transpeptidases and transglycosylases during peptidoglycan synthesis. The lipid moiety is processed and the resulting protein and peptidoglycan fragment is integrated into to the cell wall, covalently anchoring the protein to the peptidoglycan amino acid cross-bridge and exposing it to the surface (Fig. 7A) (321, 322).

Functions of MSCRAMMs in uropathogenic bacteria

The initial stage of adherence and colonization is absolutely necessary for pathogenic Gram-positive bacteria to establish successful infection (323). MSCRAMMs mediate this process by facilitating the recognition and binding of surface exposed host ligands to promote colonization of specific tissues. In the context of UTIs, Gram-positive uropathogens express and assemble specialized MSCRAMM molecules with a distinct tropism for urogenital epithelia. This tropism is dependent both on the ligand expressed by the host tissue, in most cases a component of the extracellular matrix, as well as the affinity for that ligand by the bacterial adhesin. However, compared to well-characterized Gram-negative adhesins such as type 1 and P pili, much less is known about the mechanisms of MCRAMMs during bacterial infection of the urinary tract.

Ace

E. faecalis, a major contributor of endocarditis and infections of the blood, wounds and abdomen, is also associated with high incidences of nosocomial catheter associated urinary tract infections (CAUTIs) (324, 325). The Ace A domain is required for recognition and binding of extracellular matrix molecules. *In vitro* mutational analysis as well as competitive inhibition studies of the A domain show that the protein has binding affinity for laminin and types I and IV collagen, each of which is a major component of the extracellular matrix (297). Ace has been identified as an important virulence determinant of *E. faecalis* in UTIs, using the murine infection model. Lebreton and colleagues showed that significantly higher doses of an *E. faecalis ace* deletion mutant are required to establish infection in mice when compared to wild-type *E. faecalis* (306). Furthermore, organ burden analysis revealed that an *ace* deletion mutant is attenuated in its ability to colonize renal tissue (306, 312). While current research implicates collagen as the major Ace binding ligand, further work needs to be done to determine the role of Ace and its binding partners in the establishment of UTIs.

UafA

S. saprophyticus holds significant prevalence as a causative agent of uncomplicated UTIs (326). Comparative genomic analysis between *S. aureus, S. epidermidis*, and *S. saprophyticus* revealed that *S. saprophyticus* contains the unique MSCRAMM UafA (307). Expression of *uafA* by *S. saprophyticus* promotes adherence to human bladder carcinoma cells, while deletion of the *uafA* gene causes decreased adherence (307). Furthermore, strains of *S. saprophyticus* that express UafA, in addition to other surface proteins such as SdrI and Ssp, are internalized into human bladder carcinoma cells (327). Although the precise ligand or tissue tropism of UafA remains unknown, preliminary data suggest that the ligand may be a carbohydrate or lipid molecule rather than a protein (313).

UafB

UafB is a recently discovered, plasmid-encoded MSCRAMM of *S. saprophyticus* that affects bacterial

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adherence to uroepithelial cell lines (308). In addition to a predicted N-terminal Sec signal sequence and C-terminal CWSS, the majority of the 2279 residue UafB protein comprises three serine-rich tandem repeats (repeats 1–3) and a single non-repeating region. The non-repeating region lies between repeats 1 and 2, and is a putative binding domain. The third repeat region is the longest of the three and is located just upstream of the CWSS. *S. saprophyticus* UafB is predicted to be glycosylated on the surface of *S. saprophyticus* (308). Although less prevalent than UafA among *S. saprophyticus* isolates, strains expressing UafB exhibit increased adhesion to human bladder carcinoma cells when compared to a *uafB* mutant (308). Analysis of the putative non-repeat binding domain indicates that UafB binds both fibronectin and fibrinogen, but not collagen types I, II, or IV; laminin; or vitronectin (308). This information may suggest that UafB has a tropism for bladder epithelium since human bladder carcinoma cells abundantly express fibronectin (328). However, a role for UafB during infection remains to be established, as both wild-type and *uafB* knockout strains equally colonize mouse bladders in a murine ascending UTI model (308).

SdrI

SdrI is a recently discovered MSCRAMM in *S. saprophyticus* classified by its serine-aspartate repeat (SD or Sdr) region, which is indicative of the Sdr family of MSCRAMMs. Sequence analysis of SdrI shows that it contains a C-terminal CWSS, an N-terminal A region, a B region containing two repeat domains, and the SD region (329). Initial binding experiments showed that when SdrI was deleted, *S. saprophyticus* exhibited a significant deficiency in adhesion to collagen compared to wild-type bacteria (329). Further work indicated that SdrI also has affinity for fibronectin, which is dependent on its A domain (296). This is unique since SdrI does not contain any known fibronectin binding motifs. In vivo data using a murine UTI model revealed that *sdrI* mutant *S. saprophyticus* bacteria do not have defects in initial colonization of the bladder or kidneys when compared to wild-type bacteria; however, the mutant strain is cleared faster from these organs (309). This suggests a role for SdrI in the persistence of *S. saprophyticus* in the urinary tract, rather than in initial adhesion or colonization.

Additional surface-located adhesins

A unique class of Gram-positive surface exposed adhesins has been discovered that lack both a Sec secretion signal as well as a cell wall localization LPXTG motif (330). These adhesins are not classified as MSCRAMMs or pili. While little has been elucidated as to their structure, one such adhesin, the enterococcal fibronectin-binding protein A (EfbA), expressed by *E. faecalis*, has been implicated in mouse models of ascending urinary tract infections. An *efbA* deletion strain was significantly attenuated in its ability to bind immobilized fibronectin *in vitro* as well as mouse kidneys and bladders *in vivo* (331).

An additional protein, Esp, has been implicated in colonization and persistence of *E. faecalis* in the urinary tract (332). Esp has not been classified as an MSCRAMM, but it contains an N-terminal Sec signal sequence, a central region with a non-repeat domain followed by a number of repeating sequences, and a CWSS-like region at the C terminus with a variation of the LPXTG motif. The N-terminal region of Esp promotes biofilm formation in vitro, but may do this indirectly (333).

GRAM-POSITIVE PILI

Although first described in 1968, the assembly of pili by Gram-positive bacteria has only been widely recognized and characterized since the work of Ton-That and Scheewind beginning in 2003 (17–20). Multi-subunit, peptidoglycan-linked adhesive pili have now been described on the surface of a number of Gram-positive bacteria (21, 22). Like MSCRAMMs, pili play important roles in binding to and recognizing extracellular matrix molecules, colonization of host tissues, and biofilm formation. Gram-positive pili are expressed from gene clusters encoding one or more minor pilins, a major pilin, and pilus assembly machinery in the form of pilus-specific sortases. Gram-positive pili expressed by *C. diphtheria* and Streptococcal pathogens have been well studied and serve as prototypes (334–338). Although similar to Gram-negative pili in their general ultrastructure (hair-like polymeric fibers) and functions (adhesion to and colonization of surfaces), Gram-positive pili have unique features, including the presence of intramolecular and intermolecular covalent bonds that stabilize the fibers against factors encountered in the extracellular environment. Currently, *E. faecalis and E. faecium* are the only Gram-positive bacteria known to express pili associated with UTIs. These pili were first identified as biofilm determinants during endocarditis infections and are named Ebp for endocarditis and biofilm associated pilus (339, 340).

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Structure of Gram-positive pili

The pilus fiber

Gram-positive pili form thin, extended fibers one protein subunit wide (approximately 3 nm diameter) and up to several micrometers in length. The pilus fiber is built from repeating copies of covalently linked major pilus subunits, with minor pilins covalently incorporated at different points in the fiber. Minor pilins that function to recognize and bind target host ligands are known as tip pilins, while pilins that anchor the entire pilus fiber to the cell wall are referred to as base pilins. The major pilin forms the pilus shaft, which functions to extend the adhesive tip pilin distally from the bacterial surface.

A number of crystal structures have been solved for Gram-positive pilins, including from *C. diphtheria*, Streptococcal *species*, and *Bacillus cereus* (Fig. 8) (22, 334–338, 341). Pilins share a common domain organization with MSCRAMMs, comprising an N-terminal Sec secretion signal, a central structural region, and a C-terminal CWSS (Fig. 9). The central structural region typically contains multiple N subdomains that adopt DEv-IgG or IgG-rev folds (Fig. 8) (335, 342). An important characteristic of Gram-positive pilins is their ability to form covalent, intramolecular isopeptide bonds between lysine and asparagine residues of the DEv-IgG and IgG-rev folds (Fig. 8) (343). These isopeptide bonds promote resistance to proteases and increase pilin structural stability (334). DEv-IgG folds typically possess a D-type isopeptide bond, in which a lysine residue in the A β-strand of sheet 1 forms a bond with an asparagine on the antiparallel F β-strand of sheet 2 (Fig. 8). A C β-strand aspartic acid residue on sheet 2 catalyzes this reaction. IgG-rev folds exhibit an E-type bond where a lysine on the A β-strand of sheet 1 is covalently linked to the asparagine of the parallel G β-strand, also of sheet 1 (Fig. 8). A glutamine residue on the sheet 2 E β-strand catalyzes this reaction (22).

In addition to their LPXTG motif, major pilins also have a conserved pilin motif, typically of the sequence VYPK, housing an essential lysine residue (22). An intermolecular isopeptide bond is formed between the lysine of the pilin motif and the threonine of the LPXTG motif of a neighboring subunit in the pilus fiber, catalyzed by a pilus-specific sortase (Fig. 9). Tip pilins have an LPXTG motif but typically lack a pilin motif, and therefore can only be incorporated at the beginning of the pilus fiber. Base pilins, which contain both LPXTG and pilin motifs, are linked to the peptidoglycan cell wall via their LPXTG motif by the housekeeping sortase, thus anchoring the pilus fiber to the bacterial surface (Fig. 9) (336). In some instances, base pilins can also be incorporated along the length of the pilus fiber (19, 344). Some pilins, particularly major pilins, may also possess a third motif called an E-box, which contains an invariant glutamic acid residue. Little work has been done to elucidate the role of the E-box in pilus assembly, but it may facilitate integration of minor pilins throughout the pilus fiber (19).

Ebp pili

The Ebp operon, found in both *E. faecalis* and *E. faecium*, consists of four genes, *ebpA*, *ebpB*, *ebpC*, and *srtC* on what is termed a pilus island on the bacterial chromosome (Fig. 9) (340). Little is known about the structure of Ebp pili, but studies have shown that EbpA and EbpB are minor pilins, with EbpA serving as the tip pilin and EbpB as the base pilin (340). EbpC is the major structural pilin. SrtC is a class C pilus specific sortase required for polymerization of the pilus fiber (340, 345, 346). Sequence analysis of these pilins suggests that they share structural characteristics with known MSCRAMMs, having potential Dev-IgG and IgG-rev domains (347). EbpA and EbpC also possess predicted E-box motifs (340, 347).

Sequence and mutational analysis of EbpA predicts that the tip pilin contains a von Willebrand factor A (VWA) domain with a metal ion-dependent adhesion site (MIDAS) (Fig. 8), which facilitates adhesion to host molecules (345, 348). VWA domains are found in proteins from different kingdoms and contain a β-sheet surrounded by a number of α-helices. Many VWA domain proteins also contain a MIDAS motif (348). Functional analysis of the EbpA MIDAS motif indicates that it is essential for EbpA function (348, 349). While the ligand specificity of EbpA remains unknown, other VWA domain- and MIDAS motif-containing proteins bind extracellular matrix proteins such as collagen (350).

Assembly of Gram-positive pili

The assembly mechanisms of Gram-positive pili in bacteria such as *C. diphtheriae* have been well studied and share many commonalities with the anchoring of MSCRAMMs to the cell wall (19, 20, 292). Additional pilus-specific features, including the pilin motif and class C sortases, are required for biogenesis of the multisubunit pilus fiber. The initial stages of pilus assembly are essentially the same as for all LPXTG cell wall anchored proteins. Like MSCRAMMs, Gram-positive pilins contain a cleavable N-terminal signal sequence for targeted translocation across the cytoplasmic membrane via the general Sec pathway. Following translocation,

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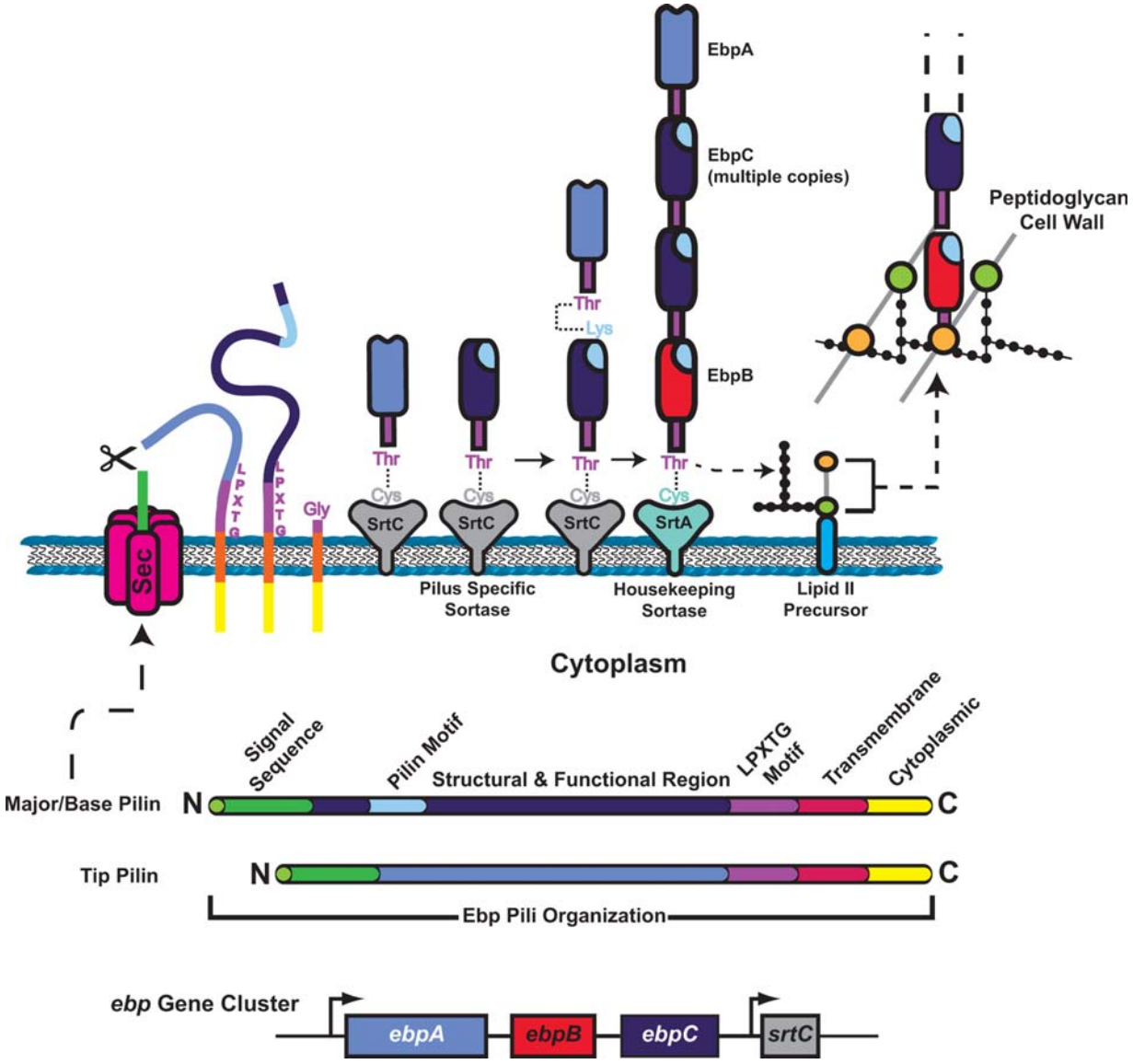

FIGURE 9 Model for Gram-positive pilus polymerization and incorporation into the cell wall. The domain organization of typical major and minor pilins is shown at the bottom, along with the *ebp* gene cluster coding for Ebp pili of *E. faecalis*. The steps of secretion across the cytoplasmic membrane and covalent linkage to a sortase are the same as for MSCRAMMs, except the pilins are processed by the SrtC pilus-specific sortase. Pilus subunits are polymerized by formation of intermolecular isopeptide bonds between the Lys of a pilin motif of one subunit and the Thr of the LPXTG motif of a preceding subunit in the fiber. Linkage to the cell wall occurs when a growing pilus fiber is transferred to a base pilin bound to the SrtA housekeeping sortase. Integration of the pilus into the cell wall follows the mechanism as described for MSCRAMMs. doi:10.1128/microbiolspec.UTI-0018-2013.f9

the transmembrane domain and the positively charged cytoplasmic tail of the CWSS retains and orients the pilin as so to make the LPXTG motif accessible for cleavage by a sortase (Fig. 9). Gram-positive pilus gene clusters typically encode a class C pilus specific sortase (srtC), which is essential for pilus polymerization (340). Differences between the pilus specific and housekeeping (SrtA) sortases determine how the LPXTG motif of individual pilins are processed, either for covalent linkage to the lipid II peptidoglycan precursor via SrtA, or for the formation of structural pilin-pilin isopeptide bonds via SrtC.

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For both major and minor pilins, SrtC cleaves between the threonine and glycine residues of the pilin LPXTG motif, forming an acyl-enzyme intermediates between the active site cysteine of the sortase and the threonine of the pilin (Fig. 9). Major pilins contain a pilin motif (VYPK) in addition to the LPXTG motif, whereas tip pilins typically only contain the LPXTG motif. To incorporate the tip pilin into the pilus fiber, SrtC catalyzes formation of an intramolecular isopeptide bond between the lysine of the pilin motif of the major pilin with the threonine of the LPXTG motif of the tip pilin (Fig. 9). This reaction releases the tip pilin from its SrtC molecule, forming a tip pilin-major pilin-SrtC complex (351). This process is then repeated to add additional major pilins to the complex, elongating the pilus structure and forming the pilus shaft to project the tip pilin distally from the bacterial surface. Pilus elongation is terminated via incorporation of a minor base pilin into the pilus structure by the same mechanism (21, 292). Following elongation, the pilus fiber must be securely anchored to the peptidoglycan cell wall. This is achieved by the SrtA housekeeping sortase, which catalyzes isopeptide bond formation between the LPXTG motif of the base pilin and a lipid II amino group (Fig. 9) (345). As for MSCRAMMs, the lipid II-pilus fiber complex is then processed by transpeptidases and transglycosylases, covalently securing the pilus to the peptidoglycan cross-bridge.

Function of Ebp pili in UTIs

The Ebp pili expressed by *E. faecalis* and *E. faecium*, two major causative agents of hospital acquired CAUTI, are the only Gram-positive pili that have been associated with UTIs (345, 352). The connection between Ebp pili-mediated biofilm formation and infection was first investigated in a rat endovascular model (340, 353). Data from this work showed that strains with deletions of each individual pilin, or a deletion of *srtC* alone, had significant defects in biofilm formation in vivo. Additionally, a deletion strain of just the tip pilin *ebpA* was significantly attenuated for colonization of aortic vegetations and kidneys, when compared to wild-type bacteria, suggesting a role for Ebp pili in UTIs as well as endocarditis (340). Later studies confirmed the role of Ebp pili in colonization of the urogenital tract, using *ebpA* and *ebpC* deletion strains to show a colonization defect in both the kidneys and bladders of mice (346, 352). Similar results were observed in experiments using *ebp* deletion mutants in *E. faecium* (339).

Experiments have also been done to identify the role of Ebp pili in CAUTI. Using a murine CAUTI model, an *E. faecalis* strain lacking the *ebpABC* pilus genes displayed significantly reduced adherence compared to the wild-type strain to bladders and silicone implants placed in the bladder to mimic catheters (345). Interestingly, bacteria lacking only the major pilin EpbC colonized mouse bladders and implants similar to wild-type bacteria, suggesting that expression of just the minor pilins on the bacterial surface is sufficient to mediate colonization. Although the ligand(s) for Ebp pili remain unknown, mutations introduced into the MIDAS motif of the EbpA tip pilin drastically inhibited bacterial adhesion in the CAUTI model, indicating a role for this motif in EbpA function and pilus-mediated adhesion (345).

CONCLUSION

The diverse array of adhesins expressed by uropathogenic bacteria reflects the importance of adhesion to colonization. Pathogens invading into the urinary tract must have strong adherence properties to overcome the washing action of the flow of urine. In addition, adhesins expressed by bacteria must be able to withstand mechanical forces exerted by urine flow, to avoid being sheared off once they have bound to their receptors on the urothelial surface. These factors underlie the prominence of pilus adhesins such as type 1 and P pili, which are adapted to function in the urinary tract, among the virulence factors of uropathogens. Uropathogenic bacteria encode multiple different types of adhesins, providing specificity for different niches within the urinary tract, as well as redundancy in function to ensure maintenance of adhesion under varying conditions. Bacterial colonization is also enhanced by formation of bacterial-bacterial interactions and biofilm structures, explaining the association of adhesins, including curli and Ag43 with uropathogens.

Adhesion is crucial at early stages of infection, and thus represents an attractive target for therapeutic intervention. Advances in understanding the structure and assembly of bacterial adhesins will be critical for the development of effective vaccines and antimicrobial agents that target adhesion. Pilus-based vaccines have shown promise in preventing UTIs, although none has reached clinical use. Studies using purified P pili or recombinant PapG adhesin purified in complex with its PapD chaperone demonstrated protection against pyelonephritis in primates (163, 354). Similarly, vaccination with the type 1 pilus adhesin FimH, purified in complex with its FimC chaperone, provided protection in primates against challenge with a UPEC cystitis isolate

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(129). Interestingly, a recent study found that rather than blocking type 1 pilus-mediated adhesion, the host antibody response may actually enhance binding of FimH to its ligands by stabilizing the adhesin's high-affinity binding state (355). An improved understanding of pilus adhesion mechanisms under conditions in the urinary tract may allow tailoring of the antigen used for vaccination to provoke a more effective immune response. In addition to pili, surface-located adhesins such as the MSCRAMMs expressed by Gram-positive bacteria also represent viable targets for vaccine development (356, 357).

An alternative approach to vaccination is to disrupt bacterial adhesion to host cells through the use of small molecule competitive inhibitors of adhesin-receptor interactions. Examples include the use of galabiose- and mannose-based inhibitors to interfere with adhesion mediated by P and type 1 pili, respectively, with the goal of preventing UPEC colonization of the urinary tract (358–361). Another alternative approach is to develop small molecule inhibitors that disrupt the machinery used for adhesin biogenesis, thereby preventing assembly of the adhesins on the bacterial surface. Once such class of small molecules developed by Almqvist and colleagues, termed pilicides, interferes with the CU assembly pathway and blocks expression of both P and type 1 pili by *E. coli* (362). Pilicides target the periplasmic chaperone and appear to disrupt pilus assembly by interfering with binding of chaperone-subunit complexes to the outer membrane usher. Modified pilicides were recently developed that also had activity against curli (203). Treatment of UPEC with one of these inhibitors blocked assembly of both curli and type 1 pili, reduced biofilm formation, and attenuated bacterial colonization in the murine urinary tract infection model (203). These compounds highlight the potential for a new class of anti-infective agents that target virulence factor secretion systems and the assembly of virulence-associated surface structures, rather than disrupting essential biological processes as for conventional antibiotics (363, 364). Such anti-virulence molecules should place less pressure on the bacteria and thus may be less prone to the development of resistance mechanisms. This strategy may also allow the selective targeting of pathogenic bacteria, avoiding detrimental side effects of broad-spectrum antibiotics on the normal bacterial flora.

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