Adhesins Involved in Attachment to Abiotic Surfaces by Gram-Negative Bacteria

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ABSTRACT During the first step of biofilm formation, initial attachment is dictated by physicochemical and electrostatic interactions between the surface and the bacterial envelope. Depending on the nature of these interactions, attachment can be transient or permanent. To achieve irreversible attachment, bacterial cells have developed a series of surface adhesins promoting specific or nonspecific adhesion under various environmental conditions. This article reviews the recent advances in our understanding of the secretion, assembly, and regulation of the bacterial adhesins during biofilm formation, with a particular emphasis on the fimbrial, nonfimbrial, and discrete polysaccharide adhesins in Gram-negative bacteria.

INTRODUCTION
The ability of bacterial cells to adhere to and interact with surfaces to eventually form a biofilm is a crucial trait for the survival of any microorganism in a complex environment. As a result, different strategies aimed at providing specific or nonspecific interactions between the bacterial cell and the surface have evolved. While adhesion to abiotic surfaces is usually mediated by nonspecific interactions, adhesion to biotic surfaces typically requires a specific receptor-ligand interaction (1). In both cases, these interactions usually originate from the same fundamental physicochemical forces: covalent bonds, Van der Waals forces, electrostatic forces, and acid-base interactions (2). Strong adhesion occurs if a bacterium and a surface are capable of forming either covalent, ionic, or metallic bonds, but weaker forces, such as polar, hydrogen bonding, or Van der Waals interactions, can also strengthen or achieve strong interactions when a high number of contacts are involved (2, 3). Due the net negative charge of their cell envelopes, bacteria are subjected to repulsive electrostatic forces when approaching surfaces. Bacterial cells also encounter repulsive hydrodynamic forces near the surface in a liquid environment. To overcome these two repulsive barriers, bacteria typically use organelles, such as flagella or pili, which act either as an active propeller or a grappling hook (4–6). Once on the surface, the cell can enhance attachment to the surface via specific and/or nonspecific adhesins to eventually trigger irreversible attachment. This irreversible attachment is strongly influenced by environmental factors (i.e., pH, salinity, etc.) and the physicochemical properties of the surface (i.e., rugosity, hydrophobicity, charge, etc.) but also by the presence of the conditioning film, a layer of organic and inorganic substances that can alter the physicochemical properties of the surface.

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inorganic contaminants adsorbed on the surface which changes its physicochemical properties (7). To achieve permanent adhesion under such variable conditions, bacterial cells have developed a series of adhesins able to facilitate adhesion under various environmental conditions (8, 9). In this article, we will focus exclusively on nonspecific adhesins, which are primarily responsible for biofilm formation and bacterial adhesion to abiotic surfaces. We will review the current knowledge of fimbrial, nonfimbrial, and discrete polysaccharide adhesins involved in adhesion to abiotic surfaces and cell aggregation in Gram-negative bacteria.

**Fimbrial Adhesins/Pili**

Fimbrial adhesins are a varied yet ubiquitous group of adhesins in both Gram-positive and Gram-negative bacteria. Also referred to as attachment pili, these polymeric fibers are involved in an array of functions, including attachment to both biotic and abiotic surfaces, motility, DNA transfer, and biofilm formation. Visible on the cell surface via electron microscopy, fimbrial adhesins are complex appendages that often require a large number of proteins for proper assembly.

The role of pili in the biofilm is multifaceted. First, they are important for the initial stage of bacterial cell attachment to both biotic and abiotic surfaces. Pili are often involved in the transition between motility and irreversible attachment, as seen with chaperone-usher pili (CUP) of *Escherichia coli* and tight adherence (Tad) pili of *Caulobacter crescentus* (10, 11). Second, they facilitate intercellular interactions through aggregation or microcolony formation, which is demonstrated with both Tad pili and curli. Finally, they also play a role in the secondary structure of the biofilm through their function in twitching motility. The type IVa pili of *Pseudomonas aeruginosa* help generate a mature biofilm structure through type IV pili–mediated migration of a key subpopulation of cells within the biofilm (12–14). Many bacterial species have more than one type of pili, and these can be divided into four subgroups, generally defined by their secretion and assembly processes: (i) CUP, (ii) type IV pili, (iii) alternative chaperone-usher pathway pili, and (iv) pili assembled by the extracellular nucleation-prefilament pathway, also known as curli. The structure and the role of Gram-negative fimbrial adhesins in biofilm formation will be discussed in this section.

**The CUP**

The CUP are the most ubiquitous type of pili and are generally made of either long and thin or heavier rod-like filaments. CUP are assembled by the concerted action of a periplasmic chaperone and a pore-forming protein, called the usher, which gives this type of pilus its name. Although best described in *Enterobacteriaceae*, CUP are also found in a variety of other Gram-negative bacteria, including *Acinetobacter* spp., *Haemophilus influenzae*, *Burkholderia cenocepacia*, *Rhizobium* spp., and *Xylella fastidiosa* (Table 1) (15–18). Based on the phylogeny of the usher protein sequences, CUP are subdivided into six main clades: α, β, γ, κ, π, and σ (19). Although there are as many as 38 different CUP in *E. coli* (20), the best studied are type I (γ1 clade) and P pili (π clade), which provide a good model for CUP assembly. Here we will use the *E. coli* type I pilus as the example for assembly (for more detail, see reference 21 or 22).

The type I pili consist of six subunits that are first translocated into the periplasm via the Sec translocation pathway (Fig. 1A). After crossing the inner membrane, the pilus subunits associate with a periplasmic chaperone, FimC, which facilitates association with FimD, the outer membrane usher protein. FimD, an 800–amino acid β-barrel protein, is referred to as the type I pilus assembly platform. The pilus subunit, chaperone, and usher proteins participate in a process called donor strand exchange and donor strand complementation to facilitate pilus filament assembly (21). Briefly, each pilus subunit has the structure of an incomplete immunoglobulin fold such that it is missing a C-terminal β-strand within the fold. Each subunit also contains an N-terminal extension. During folding and association with the chaperone subunit (FimC), the chaperone complements the missing β-strand within the pilus subunit with hydrophobic residues from the G1 strand of the chaperone. Then, the chaperone (FimC) and the pilus subunit associate with the usher (FimD) (Fig. 1A). The N-terminal extension of the pilus subunit already bound to FimD moves into the groove of the pilus subunit occupied by the chaperone (FimC) G1 strand. The donor strand exchange occurs subsequently by a zip-in zip-out mechanism to mediate transfer of the incoming pilus subunit onto the growing pilus rod. The chaperone (FimC) is then recycled to bind newly formed pilus subunits (Fig. 1A). The initiation of pilus biogenesis occurs when the FimC-FimH complex associates with the usher FimD. Elongation begins with the arrival of the FimC-FimG complex. FimH and FimG comprise the 3-nm tip fibrillum, a small fiber associated with the tip of the main pilus (Fig. 1A). Then, FimF is connected to the tip adhesin and subsequently initiates assembly of the body of the main rod that is composed of FimA (Fig. 1A). P pilus
assembly is then terminated with the addition of PapH to the proximal end of the pilus. PapH is unable to undergo donor-strand exchange and thus terminates pilus growth. By comparison, no homologues of PapH have been identified in the type I pilus assembly.

The *E. coli* type I pilus is composed of 500 to 3,000 copies of FimA, resulting in a fiber that is 1 to 2 μm long and 6.9 nm wide. The absence of the tip adhesin FimH reduces adherence and biofilm formation, suggesting that FimH is the business end of the type I pilus (10). FimH is specific for mannose-containing receptors of host cells but can also bind to abiotic surfaces in a mannose-dependent manner (10, 13). Mannose was found to specifically inhibit biofilm formation in *E. coli* on a variety of abiotic surfaces, including various plastic polymers and glass (10). This specificity resulted in the development of mannose drug analogs to inhibit bacterial attachment (23). In addition to being involved in attachment to abiotic surfaces, FimH primarily mediates strong adherence to mannose receptors using catch bonds, a type of bond that becomes stronger over time through repeated pulling apart (24, 25). Catch bonds are particularly effective for organisms that experience shear stress, such as those that colonize host mucosal surfaces or air-water interfaces. A variety of other CUP have been reported to play a role in biofilm formation (Table 1).

### The Alternative CUP

The alternative CUP (or class 5 pilus assembly) requires “usher” and “chaperone” proteins and has been classified in the α-clade of the CUP family of fimbriae (19). However, the proteins in the alternative CUP pathway have little to no similarity to those in the CUP pathway, and only four proteins are known to be required for proper assembly of the alternative CUP (19). This clade includes several *coli* surface (CS) antigens (CS) of enterotoxigenic *E. coli* (ETEC), which are involved in the intestinal epithelium colonization or in biofilm formation on indwelling devices such as bladder catheters (26), and the cable pili of *Burkholderia cepacia* that facilitate bacterial aggregation, microcolony formation, and association with mucin, a prerequisite in the development of cystic fibrosis (Table 1) (27). In this section,

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**Table 1** Examples of fimbrial adhesins involved in biofilm formation

<table>
<thead>
<tr>
<th>Pili type</th>
<th>Major pilus proteins</th>
<th>Minor proteins and assembly proteins</th>
<th>Bacteria</th>
<th>Reference</th>
</tr>
</thead>
<tbody>
<tr>
<td>Chaperone/usher</td>
<td>EcpA or MatA (ECP pilus)</td>
<td>EcpC, EcpD, EcpE, FimC, FimD, FimF, FimG, FimH</td>
<td><em>E. coli</em></td>
<td>196</td>
</tr>
<tr>
<td></td>
<td>FimA (type I)</td>
<td></td>
<td><em>E. coli, Klebsiella pneurniae,</em></td>
<td>21, 197–200</td>
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<tr>
<td></td>
<td></td>
<td></td>
<td><em>X. fastidiosa, Enterobacter amyllovora,</em></td>
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<tr>
<td></td>
<td></td>
<td></td>
<td><em>Serratia marcescans</em></td>
<td></td>
</tr>
<tr>
<td></td>
<td>CsuA/B (type I)</td>
<td>CsuC, CsuD, CsuE, MrkA, MrkC, MrkD</td>
<td><em>Acinetobacter baumannii</em></td>
<td>201</td>
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<tr>
<td></td>
<td>MrkA (type 3)</td>
<td></td>
<td><em>K. pneumoniae, E. coli (UPEC), Citrobacter koseri</em></td>
<td>202, 203</td>
</tr>
<tr>
<td>Type IV pilin</td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Type IVa</td>
<td>PlaA</td>
<td>PlaE, PlaD, PlaI, PlaW, PlaX</td>
<td><em>P. aeruginosa</em></td>
<td>14, 45, 46</td>
</tr>
<tr>
<td></td>
<td>PlaE</td>
<td>PlaD, PlaH, PlaI, PlaJ, PlaK, PlaM, ComP, PlaD</td>
<td>Neisseria spp.</td>
<td>204, 205</td>
</tr>
<tr>
<td>Type IVb</td>
<td>BtpA (bundle forming)</td>
<td>BtpP, BtpI, BtpJ, BtpK</td>
<td><em>V. parahemolyticus</em></td>
<td>49, 207</td>
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<tr>
<td></td>
<td>TcaP (Tca)</td>
<td>TcaP, C, D, E, F, TcaP</td>
<td><em>E. coli</em> (EPEC)</td>
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<td></td>
<td></td>
<td></td>
<td><em>C. crescentus</em></td>
<td>11, 66</td>
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<td></td>
<td></td>
<td><em>P. aeruginosa</em></td>
<td>56, 209</td>
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<tr>
<td></td>
<td></td>
<td></td>
<td><em>Agrobacterium tumefaciens</em></td>
<td>252</td>
</tr>
<tr>
<td>Alternative CU</td>
<td>CooA (CSI)</td>
<td>CooB, CooC, CooD</td>
<td><em>E. coli</em> (ETEC)</td>
<td>26, 28</td>
</tr>
<tr>
<td></td>
<td>CblA (cable pilus)</td>
<td>CblB, CblC, CblD</td>
<td><em>Burkholderia cepacia complex</em></td>
<td>36</td>
</tr>
<tr>
<td>Nucleation/precipitation</td>
<td>CsgA(Curli)</td>
<td>CsgB, CsgG, CsgE, CsgF, CsgD</td>
<td><em>E. coli, Enterobacter cloacae,</em></td>
<td>72, 77</td>
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<td></td>
<td></td>
<td></td>
<td><em>Citrobacter spp.</em></td>
<td></td>
</tr>
<tr>
<td></td>
<td>AgfA (Tafi)</td>
<td>AgfB, AgfC, AgfD, AgfE, AgfF</td>
<td><em>Salmonella enteritidis</em></td>
<td>210, 211</td>
</tr>
<tr>
<td></td>
<td>FapC</td>
<td>FapA, FapB, FapD, FapE, FapF</td>
<td><em>Pseudomonas spp.</em></td>
<td>212</td>
</tr>
</tbody>
</table>
Assembly and secretion of fimbrial adhesins. All the assembly pathways are oriented such that the inside of the cell is at the top and the surface to which the adhesin is binding, represented by the thick black line, is at the bottom. The subunits for the three described systems are believed to be transported across the inner membrane by the Sec machinery. (A) A schematic of the CUP pathway represented by the assembly of the E. coli type I pilus. FimC (green moon) is a chaperone. FimD (blue-gray) is the outer membrane usher shown as a dimeric channel. FimA (blue bean) is the main pilus subunit. FimF (orange bean) links the tip fibrillum to the main fiber. FimG (yellow bean) is the tip fibrillum. FimH (red bean) is the mannose-specific tip fibrillum adhesin. (B) A schematic of the alternative chaperone-usher pathway using the E. coli CS1 pilus as a model. CooB (green moon) is the chaperone. CooC (blue-gray) is the outer membrane usher. CooA (blue bean) is the main pilus subunit. CooD (red circle) is the pilus tip adhesin. (C) Model of E. coli curlin assembly as a nucleation-precipitation pathway model. CsgE (green moon) is the chaperone. CsgG (blue-gray) is the outer membrane usher. CsgA (blue beans) is the main curlin subunit. CsgF (dark blue bean) is the minor curlin subunit. CsgC (red ball) is the outer membrane protein needed for curlin polymerization and CsgB localization. CsgC (red ball) may be important for CsgG localization. Abbreviations: IM, inner membrane; CW, cell wall; OM, outer membrane. doi:10.1128/microbiolspec.MB-0018-2015.f1
the CS antigen 1 (CS1) pilus of ETEC will serve as our model (Fig. 1B) (Table 1) (for more detail on the alternative CUP pathway see references 28–30).

It is believed that the CS1 pilus proteins rely on the Sec pathway for translocation across the inner membrane, as also observed for the proteins of the CUP system. The 15.2-kDa CooA is the major pilin subunit. CooA can spontaneously form multimers in the periplasm, but the multimerization is greatly improved in the presence of CooB, which acts as a chaperone for CooA pilin subunits (Fig. 1B) (28, 31, 32). CooC is a 94-kDa integral outer membrane protein (33) predicted to be the usher protein required for transport and assembly of the pili on the cell surface (Fig. 1B). CooD is a 38-kDa minor pilin associated with the tip of the CS1 pilus (Fig. 1B) (33). The ratio of CooD to CooA is 1:1,800, indicating that one CooD could be present at the tip of the pilus. CS1 pilis are not assembled in the absence of CooD, suggesting that CooD could initiate pilus assembly as observed for the FimH tip adhesin (34). Similar to the CUP pilin subunits, the pilin of the alternative CUP pathway also undergoes donor strand exchange to facilitate transfer of the pilin subunits from the chaperone to the growing pilus fiber.

ETEC is a leading cause of diarrhea in children in developing countries. These bacteria use a variety of surface adhesins to bind to the intestinal epithelium (35). ETEC can also form biofilms on indwelling devices such as bladder catheters. Both an ETEC CS2 cotD mutant and a colonization factor antigen II (CFA/II) cfaE mutant had reduced biofilm formation relative to wild-type parental strains (26), suggesting that alternative CUP are important for adhesion.

B. cenocepacia is an important pathogen associated with cystic fibrosis. The cable pili of B. cenocepacia facilitate bacterial cell-cell interactions or microcolony formation and association with mucin (Table 1) (27). However, these cell-cell interactions actually prevent nonspecific aggregation that can result in clearance by the host. A pilin subunit (cblA) mutant had increased aggregation relative to wild-type (36).

**Type IV Pili**

The type IV pili are multifunctional organelles involved in diverse functions such as bacterial twitching motility, auto-aggregation, attachment, and DNA uptake (37). They are generally long thin filaments (6 to 8 nm wide and 1 to 4 μm long) composed of pilin subunits that in some cases assemble into bundles.

The type IV pili are often homopolymers of a 15- to 20-kDa pilin protein, and they can also have an additional adhesive tip subunit. Both the CUP and type IV pilus assembly requires the general secretory pathway to translocate subunits across the inner membrane as well as a variety of outer membrane components to assemble the pilus on the cell surface. However, by comparison with CUP, type IV pili also require additional inner membrane components to facilitate fiber assembly. The dynamic assembly/disassembly of type IV pilus into a fiber is powered by ATP and requires at least 10 proteins: (i) the major pilin subunit and sometimes minor pilin subunits, (ii) an assembly-specific ATPase, (iii) a prepilin peptidase that cleaves the N-terminal signal peptide, (iv) an assembly platform, (v) an outer membrane protein that recruits the ATPase, and (vi) an outer membrane secretin that is necessary for the presentation of the pili on the cell surface (Fig. 2) (13). Based on the length of the prepilin signal peptides, type IV pili can be subdivided into two categories: type IVa, characterized by prepilins with a short signal peptide (5 to 10 amino acids) and type IVb, characterized by prepilins with a long signal peptide (15 to 30 amino acids) (13). In this section we will focus on individual examples of type IVa, type IVb, and a subset of type IVb called the Tad pili.

**Type IVa Pili**

We will use P. aeruginosa as a model for type IVa pilus assembly and secretion (Fig. 2, Tables 1 and 2) (for more detail see reference 14). The pilus itself is primarily composed of the 15-kDa main pilin PilA (Fig. 2) and the minor pilins FimU, PilV, PilW, PilX, and PilE. All the pilin subunits have signal peptides and are processed by PilD, a prepilin peptidase, which cleaves pilin signal peptides and is bound to the inner membrane. The minor pilins are incorporated throughout the pilus structure and are important in the initiation of pilus assembly (38). There have been several other roles suggested for the minor type IVa pilins. PilX, PilY1, and PilW have been shown to be involved in cyclic diguanosine monophosphate (c-di-GMP)–mediated suppression of swarm- ing motility and biofilm formation which is facilitated through a decrease in cellular c-di-GMP levels in cells grown on an agar surface compared to liquid medium (39). In addition, PilY1 is needed for both twitching and swarming motility and facilitates attachment to host cells in a calcium-dependent manner (40). The inner membrane components of the type IVa pilus include the motor and alignment subcomplexes. The motor complex is composed of PilB, PilC, PilT, and PilU (Fig. 2, Table 2). PilB, PilT, and PilU are putative ATPases required for either pilus assembly (PilB) or disassembly (PilT and PilU). PilC is an integral inner

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**Table 1**

<table>
<thead>
<tr>
<th>Pilin</th>
<th>CUP Pathway</th>
<th>Function</th>
</tr>
</thead>
<tbody>
<tr>
<td>CooA</td>
<td>Multimerization</td>
<td>Chaperone</td>
</tr>
<tr>
<td>CooB</td>
<td>Multimerization</td>
<td>Chaperone</td>
</tr>
<tr>
<td>CooC</td>
<td>Multimerization</td>
<td>Chaperone</td>
</tr>
<tr>
<td>CooD</td>
<td>Tip Adhesion</td>
<td>Tip Subunit</td>
</tr>
</tbody>
</table>

**Table 2**

<table>
<thead>
<tr>
<th>Pilin</th>
<th>CUP Pathway</th>
<th>Function</th>
</tr>
</thead>
<tbody>
<tr>
<td>PilA</td>
<td>Multimerization</td>
<td>Chaperone</td>
</tr>
<tr>
<td>PilB</td>
<td>Assembly</td>
<td>ATPase</td>
</tr>
<tr>
<td>PilD</td>
<td>Multimerization</td>
<td>Prepilin Peptidase</td>
</tr>
<tr>
<td>PilU</td>
<td>Assembly</td>
<td>ATPase</td>
</tr>
</tbody>
</table>

**Fig. 1B**

Visual representation of the type IV pilus assembly process.

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**Fig. 2**

Diagram showing the components of the type IV pilus assembly system.

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**Fig. 2, Tables 1 and 2**

Tables showing the components of the type IV pilus assembly system.
membrane protein thought to play a role in regulation of pilus assembly and depolymerization (41). The alignment complex bridges the inner membrane motor and the outer membrane secretion complex and is composed of PilM, PilN, PilO, and PilP (Fig. 2). PilM is an actin-like cytoplasmic protein that is associated with PilN, an inner membrane protein (42). PilN also associates with PilO and the lipoprotein PilP to subsequently interact with PilQ, the outer membrane secretin. PilN also associates with FimV, a peptidoglycan binding protein that affects the multimerization of PilQ in the outer membrane (Fig. 2) (43). Finally, PilQ forms a large 1-MDa multimeric secretin that facilitates the presentation of the pilus on the cell surface. PilQ is associated with PilF via several tetratricopeptide repeats. PilF is an outer membrane lipoprotein required for the proper localization of PilQ in the outer membrane and is referred to as a pilotin (44).

While type IVa pili are usually associated with twitching motility, they also play an important role in attachment and biofilm structure. Twitching motility or crawling involves the ability of bacteria to move over semisolid surfaces using type IV pili. Cells extend individual pili that attach to a surface and then retract the pili, pulling the cell forward (14). Type IVa pilus-mediated twitching motility of P. aeruginosa is important for not only the initial attachment of the bacteria, but also for the formation of microcolonies and three-
dimensional structures in the biofilm architecture (45, 46). Hyperpiliated strains of P. aeruginosa, where twitching motility is inhibited, form dense flat monolayers (47).

Type IVa pili, and more specifically the MSHA (mannose-sensitive hemagglutinating) pilus of Vibrio cholerae and Vibrio parahaemolyticus facilitate proper biofilm formation on biotic and abiotic surfaces, including both chitin and nonchitinaceaeous surfaces, but remain dispensable for the process of initial attachment (48, 49).

Type IVb pili

While most type IVa pili are associated with twitching motility, the type IVb and Tad pili are more often associated with attachment, since the PilT ATPase homologue required for pilus retraction is absent. There are seven type IVb pili that have been characterized in Gram-negative bacteria, including the Tad pili. To examine the structure and assembly of type IVb pili, we will look at the V. cholerae toxin coregulated pilus (TCP) (Tables 1 and 2). TCP plays a role in microcolony formation and mediates bacterial interactions on chitinaceous surfaces (50) that are abundant as part of shellfish in aquatic environments. The TCP results in a more stable and fit biofilm on chitin-rich surfaces (50). V. cholerae can degrade chitin and utilize it as a source of food, which can facilitate survival and spread of V. cholerae in the environment (51).

To be properly assembled, TCP require about 10 to 14 genes that are usually organized into one long operon called the TCP locus (52). TcpA and TcpB are the major and minor pilin of TCP pili, respectively (Table 2). TcpC is an outer membrane secretin interacting with a small protein, TcpQ, which provides proper TcpC localization and stability (53). TcpJ is the prepilin peptidase, belonging to the aspartic acid protease family, which cleaves the prepilin signal sequence between a charged and hydrophobic region within the N-terminus. The type IVb prepilin signal sequences are approximately 25 residues (Fig. 2) (54). TcpT, the putative ATPase required for pili biogenesis, is localized to the inner membrane in a TcpR-dependent manner (Table 2) (55).

Tad pili

The Tad loci of bacteria are a subset or distinct clade of the type IVb pili and are found in both Gram-negative and Gram-positive bacteria. Tad pili are very important for initial adhesion, cellular aggregation, and biofilm formation (56). Due to their small size, pili of the Tad system are also referred to as Flp (fimbrial-low-molecular weight protein) (Table 2) (56). We will review the Flp pili of Aggregatibacter actinomyctemcomitans as an example of Tad pili. Many proteins of the tad loci in A. actinomycetemcomitans share similarities with proteins involved in the type II, type III, and type IV secretion pathways (Flp1, TadV, RcpA, TadA, TadB, TadC, TadE, and TadF), but some of the Tad proteins (RcpB, RcpC, TadZ, TadD, and TadG) remain exclusively associated with the Tad system (Table 2). The pilin Flp1 is the major structural component of the Flp pili (57). TadE and TadF are important for Flp pili biogenesis (58) but are classified as pseudopilins, because they have conserved prepilin processing sites and have never been found associated with the pilus (59). Thus far, it is believed that Tad pili are not retracted, because no homologue of PilT, the ATPase required for pilus retraction, has been identified (Table 2). As observed for the P. aeruginosa type IVa ATPase PilB, the putative

**TABLE 2** Type IV pilus components

<table>
<thead>
<tr>
<th>Bacteria</th>
<th>Type IVa</th>
<th>Type IVb</th>
<th>Tad or Flp</th>
</tr>
</thead>
<tbody>
<tr>
<td>Components</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Pilin subunit</td>
<td>PilA</td>
<td>TcpA</td>
<td>Flp-1</td>
</tr>
<tr>
<td>Minor pilins</td>
<td>FimU, PilV, PilW, PilX, PilE</td>
<td>TcpB</td>
<td>Flp-2, TadE, TadF</td>
</tr>
<tr>
<td>Prepilin peptidase</td>
<td>PilD</td>
<td>TcpJ</td>
<td>TadV</td>
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<tr>
<td>Assembly ATPase</td>
<td>PilB</td>
<td>TcpT</td>
<td>TadA</td>
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<tr>
<td>Retraction ATPase</td>
<td>PilT/PilU</td>
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<td></td>
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<td>Secretin</td>
<td>PilQ</td>
<td>TcpC</td>
<td>RcpA</td>
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<td>Platform proteins</td>
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<td>Pilotin</td>
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<td>TadD</td>
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<tr>
<td>Alignment complex</td>
<td>PilM, PilN, PilO, PilP, FimV</td>
<td>TcpD, TcpR, TcpF</td>
<td>RcpB, RcpC</td>
</tr>
<tr>
<td>Secreted proteins</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Localization proteins</td>
<td></td>
<td></td>
<td></td>
</tr>
</tbody>
</table>

^NF, not found
ATPase TadA forms hexomeric complexes to power pilus assembly (60). The localization of TadA is facilitated by TadZ, a cytoplasmic multidomain protein localized to the cell pole (61). TadB and TadC share similarity with PilC-like proteins and may associate to facilitate the passage of other Tad components across the inner membrane. RcpA is strongly suspected to form a channel required for the translocation of the pilus to the cell surface, but the ability of RcpA to function as a secretin is not well characterized (62, 63) (Table 2). RcpB is an outer membrane protein that may function as a gating protein (63). TadD is a predicted lipoprotein and may be important for the assembly and targeting of the other outer membrane proteins (63).

*A. actinomycetemcomitans* is a strong biofilm former on a variety of surfaces and is a causative agent of infective endocarditis. The Tad pili of *A. actinomycetemcomitans* form bundles of fibers about 5 nm in diameter, composed of the 6.5-kDa main pilin Flp1, which are involved in adhesion to abiotic surfaces (58). The lack of processing of pre-Flp1, pre-TadE, or pre-TadF or loss of pili results in decreased cell aggregation and biofilm formation (59, 64, 65).

Unlike *A. actinomycetemcomitans*, the Tad pili in *C. crescentus* are not bundled but are expressed as individual pili at the flagellar pole (66). The Tad pili in *C. crescentus* are important for the initiation of adherence and biofilm formation (Table 1). They are crucial for the initial reversible attachment to surfaces in conjunction with flagella since the loss of pili decreases initial adherence by about 50% (67). In addition, pili are involved in surface contact stimulation of holdfast secretion. Holdfast is the powerful polysaccharide adhesin responsible for irreversible adhesion and biofilm formation, as described below (see “Holdfast” below) (68). Pili also play an important role in biofilm formation and maturation, because they are responsible for the integrity of the biofilm architecture (11).

*P. aeruginosa* makes a Tad or Flp pilus in addition to type IVa and type IVb pili (Table 1). The Flp pilus of *P. aeruginosa* has also been shown to be involved in biofilm formation and facilitates attachment to epithelial cells (69).

The plant pathogen *Agrobacterium tumefaciens* has a Tad pils encoded by the *ctp* locus. Mutations in *ctpA*, *ctpB*, or *ctpG* resulted in decreased adherence on abiotic surfaces in both short-term assays and biofilm formation relative to wild type. However, no difference was readily observed between a *ctpA* mutant and wild type during root biofilm formation (252).

**The Nucleation-Precipitation Pili**

The final group of fimbrial adhesins is assembled via the nucleation-precipitation pathway. The curli of *E. coli* and *Salmonella* are the best characterized (Table 1). This group of adhesins is covered in depth in reference 251, so it will only be touched on briefly here. Curli are members of a growing group of proteins called functional amylloids. Aggregation is the basis of amyloid formation. A protein is in an “amyloid state” when it forms bundles of fibers made of β-sheets. Uncontrolled aggregation of proteins can result in damage or disease, such as may be the case in Alzheimer’s disease. Functional amyloids in bacteria arise from aggregation producing an ordered β-sheet structure in a controlled process used to generate fibrils, spore coats, or surface coats (70). Curli systems are present in a wide variety of bacteria (71). Curli are thin fimbriae (6 to 12 nm wide) with variable lengths (72). The main curli monomer is CsgA, which when secreted via CsgG, self-assembles into fibers on the cell surface in association with the minor curlin nucleator CsgB (Fig. 1C). CsgE and CsgF function as chaperones, and CsgD is a positive regulator of the curli system. For an in-depth understanding of curli biogenesis and regulation, see reference 251 or the review by Evans and Chapman (73).

Curli are associated with both initial adherence and biofilm formation in Shigatoxin-producing *E. coli* (74). Gastrointestinal commensal *E. coli* express curli that were shown to contribute to biofilm formation (75). The curli and cellulose of enterohemorrhagic *E. coli* and ETEC work in concert in adherence and biofilm formation (76). As a consequence of curli protein self-assembly on the cell surface, cross-seeding indicates that curli can facilitate multispecies biofilm formation (77).

**NONFIMBRIAL ADHESINS**

Unlike the long, polymeric adhesins discussed above, the nonfimbrial adhesins are short monomeric or trimeric structures. This type of adhesin is widely spread among bacteria and is involved in cell attachment to abiotic surfaces and/or host cells. In this section, only nonfimbrial adhesins involved in biofilm formation will be presented. The different roles in nonspecific adhesion played by these adhesins will be discussed: nonfimbrial adhesins are directly anchored to the outer cell membrane via covalent or noncovalent interactions and, due
to their relatively short size, are usually involved in close contact between the bacterial cell and the substrate. Nonfimbrial adhesins are also involved in cell-cell interactions and aggregation. Furthermore, this type of adhesin is also shown to interact with various components of the biofilm extracellular matrix, linking the bacteria to the matrix and maintaining the biofilm architecture.

Although highly diverse in terms of structure and/or adhesive properties, nonfimbrial adhesins in Gram-negative bacteria are usually grouped into two main categories: the nonfimbrial adhesins secreted through a type 1 secretion system (T1SS) and the nonfimbrial adhesins secreted through one of the type 5 secretion systems (T5SSs). Detailed examples of nonfimbrial adhesins from both categories will be discussed in this section (for more detail on nonfimbrial adhesins see references 78 and 79).

Adhesin Secreted by the T1SS

In Gram-negative bacteria, a very large group of nonfimbrial adhesins is secreted by the T1SS. This secretion system is one of the simplest described to date in Gram-negative bacteria. It is a heteromeric complex composed of three components: (i) an inner membrane ABC (ATP binding cassette) transporter, (ii) a membrane fusion protein in the periplasmic space, and (iii) an outer membrane pore (Fig. 3). These three proteins interact and form a channel that specifically transports T1SS substrates through the bacterial envelope in a single step, directly from the cytoplasm to the extracellular space. Proteins targeted by the T1SS share a C-terminal domain of lightly conserved secondary structure that is not cleaved off during the secretion process. Proteins are secreted in an unfolded state and first interact with the ABC transporter, triggering a conformational change of the channel and subsequent ATP hydrolysis, followed by the transport of the protein through the entire secretion system and subsequent folding of the secreted protein (reviewed in reference 80).

The most studied example of nonfimbrial adhesins secreted via T1SS is the Bap (biofilm-associated protein) family of proteins. The adhesins belonging to this group are high molecular weight multidomain proteins and contain a core domain of long repeated units of variable length and sequence. All Baps share three distinct features (Fig. 4): (i) an N-terminal secretion signal for transport from the cytoplasm to the periplasm, (ii) a core domain composed of highly repeated motifs, and (iii) a glycine-rich C-terminal domain. The first Bap was identified in Staphylococcus aureus (81), and since then, numerous other Bap family members have been shown to be involved in cell adhesion to abiotic surfaces and
biofilm formation in both Gram-positive and Gram-negative bacteria (for a review, see reference 82). A computational study revealed that large adhesins sharing the Bap family features are widespread among bacteria (83). However, only a few of these proteins have been characterized experimentally. Table 3 shows the Bap proteins that have been reported to be involved in biofilm formation by Gram-negative bacteria.

In *P. fluorescens* and *P. putida*, the Bap protein LapA (large adhesion protein A) is crucial for biofilm formation (84, 85). This surface-associated protein, the largest one expressed by both organisms, is responsible for the transition between reversible adhesion via a single pole to irreversible adhesion along the entire cell length (84, 85). Indeed, lapA or the ABC transporter for LapA (required for the export of LapA to the cell surface) mutants is able to first attach to surfaces via their pole but fails to undergo later irreversible adhesion and to develop the typical biofilm architecture (85). LapA is conserved between many *P. fluorescens* and *P. putida* strains, but the length of the protein in different strains is highly variable due to the flexible number of amino acid repeats (86). LapA mediates cell adhesion to a wide array of abiotic surfaces, from various plastics to glass or quartz, suggesting that the interactions between this protein and the surface are nonspecific (84, 85). Single cell force spectroscopy experiments recently showed that different domains of LapA are involved in different adhesion processes: while the repeated units in the core domain are mainly responsible for adhesion on hydrophobic surfaces, the C-terminal domain is involved in adhesion to hydrophilic surfaces, allowing LapA adhesion to a wider range of substrates and increasing the versatility of *P. fluorescens* colonization in diverse environments (87). In addition, single cell force spectroscopy analysis of the footprint left behind by *P. fluorescens* cells detached from a surface reveals a local accumulation of LapA occurring at the cell-surface interface and the presence of multiple adhesion peaks with extended rupture lengths, highlighting the critical role of LapA in mediating the irreversible cell adhesion to surfaces (88).

Another large adhesion protein found in *P. putida* that participates in biofilm formation is LapF (89). LapF is a surface-associated protein and shares the key features of Baps (89). While LapA is responsible for irreversible adhesion of single cells to the surface, LapF is believed to mediate the cell-cell interaction in the biofilm and to play a major role during later development.
### TABLE 3

Selected examples of nonfimbrial adhesins experimentally shown to be involved in biofilm formation by Gram-negative bacteria

<table>
<thead>
<tr>
<th>Protein</th>
<th>Organism</th>
<th>Size (aa)</th>
<th>Reference</th>
</tr>
</thead>
<tbody>
<tr>
<td>LapA</td>
<td><em>Pseudomonas putida</em></td>
<td>8,682</td>
<td>84</td>
</tr>
<tr>
<td>BapA / AdhA</td>
<td><em>B. cenocepacia</em></td>
<td>2,924</td>
<td>213</td>
</tr>
<tr>
<td>LapA</td>
<td><em>Pseudomonas fluorescens</em></td>
<td>4,920</td>
<td>85</td>
</tr>
<tr>
<td>BapA</td>
<td><em>S. enterica</em></td>
<td>3,825</td>
<td>214</td>
</tr>
<tr>
<td>YeeJ</td>
<td><em>E. coli</em></td>
<td>2,358</td>
<td>102</td>
</tr>
<tr>
<td>LapF</td>
<td><em>P. putida</em></td>
<td>6,310</td>
<td>89</td>
</tr>
<tr>
<td>BfpA</td>
<td><em>Shewanella oneidensis</em></td>
<td>2,768</td>
<td>216</td>
</tr>
<tr>
<td>MRP</td>
<td><em>Pectobacterium atrosepticum</em></td>
<td>4,558</td>
<td>217</td>
</tr>
<tr>
<td>BfpA</td>
<td><em>Shewanella putrefaciens</em></td>
<td>4,220</td>
<td>218</td>
</tr>
<tr>
<td>Cat-1</td>
<td><em>Psychrobacter articus</em></td>
<td>6,715</td>
<td>219</td>
</tr>
<tr>
<td></td>
<td><strong>Monomeric autotransporter adhesins – T5SS</strong></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Ag43</td>
<td><em>E. coli</em></td>
<td>1,039</td>
<td>104</td>
</tr>
<tr>
<td>Cah</td>
<td><em>E. coli</em></td>
<td>2,850</td>
<td>220</td>
</tr>
<tr>
<td>AIDA</td>
<td><em>E. coli</em></td>
<td>1,237</td>
<td>107</td>
</tr>
<tr>
<td>TibA</td>
<td><em>E. coli</em></td>
<td>989</td>
<td>221</td>
</tr>
<tr>
<td>Yfa/L/EhaC</td>
<td><em>E. coli</em></td>
<td>1,250</td>
<td>102</td>
</tr>
<tr>
<td>YpjA/EhaD</td>
<td><em>E. coli</em></td>
<td>1,526</td>
<td>102</td>
</tr>
<tr>
<td>YcgV</td>
<td><em>E. coli</em></td>
<td>955</td>
<td>102</td>
</tr>
<tr>
<td>Hap</td>
<td><em>H. influenzae</em></td>
<td>1,392</td>
<td>127</td>
</tr>
<tr>
<td>EhaA</td>
<td><em>E. coli</em></td>
<td>1,328</td>
<td>222</td>
</tr>
<tr>
<td>Ehab</td>
<td><em>E. coli</em></td>
<td>980</td>
<td>223</td>
</tr>
<tr>
<td>UpaH</td>
<td><em>E. coli</em></td>
<td>2,845</td>
<td>224</td>
</tr>
<tr>
<td>UpaC</td>
<td><em>E. coli</em></td>
<td>996</td>
<td>225</td>
</tr>
<tr>
<td>Upal</td>
<td><em>E. coli</em></td>
<td>1,254</td>
<td>226</td>
</tr>
<tr>
<td>MisL</td>
<td><em>S. enterica</em></td>
<td>955</td>
<td>227</td>
</tr>
<tr>
<td></td>
<td><strong>Trimeric autotransporter adhesins – T5SS</strong></td>
<td></td>
<td></td>
</tr>
<tr>
<td>YadA</td>
<td><em>Yersinia pseudotuberculosis</em></td>
<td>434</td>
<td>119</td>
</tr>
<tr>
<td>UsPA1</td>
<td><em>Moraxella catarrhalis</em></td>
<td>955</td>
<td>228</td>
</tr>
<tr>
<td>Hap/MID</td>
<td><em>M. catarrhalis</em></td>
<td>2,090</td>
<td>228</td>
</tr>
<tr>
<td>UpaG</td>
<td><em>E. coli</em></td>
<td>1,779</td>
<td>120</td>
</tr>
<tr>
<td>SadA</td>
<td><em>S. enterica</em></td>
<td>1,461</td>
<td>121</td>
</tr>
<tr>
<td>AtaA</td>
<td><em>Acinetobacter sp. Tol5</em></td>
<td>3,630</td>
<td>229</td>
</tr>
<tr>
<td>EhaG</td>
<td><em>E. coli</em></td>
<td>1,589</td>
<td>230</td>
</tr>
<tr>
<td>BfbA</td>
<td><em>Burkholderia pseudomallei</em></td>
<td>1,527</td>
<td>122</td>
</tr>
<tr>
<td></td>
<td><strong>Hemagglutin-like adhesins – T5SS</strong></td>
<td></td>
<td></td>
</tr>
<tr>
<td>HxFB</td>
<td><em>X. fastidiosa</em></td>
<td>3,376</td>
<td>231</td>
</tr>
<tr>
<td>HxFA</td>
<td><em>X. fastidiosa</em></td>
<td>3,458</td>
<td>231</td>
</tr>
<tr>
<td>HMW1</td>
<td><em>H. influenzae</em></td>
<td>1,536</td>
<td>127</td>
</tr>
<tr>
<td>HMW2</td>
<td><em>H. influenzae</em></td>
<td>1,477</td>
<td>127</td>
</tr>
<tr>
<td>XadA</td>
<td><em>X. fastidiosa</em></td>
<td>763</td>
<td>125</td>
</tr>
<tr>
<td>YapH</td>
<td><em>Xanthomonas fuscans</em></td>
<td>3,397</td>
<td>124</td>
</tr>
<tr>
<td>FhaB</td>
<td><em>X. fuscans</em></td>
<td>4,490</td>
<td>124</td>
</tr>
<tr>
<td>XacFhaB</td>
<td><em>Xanthomonas axonopodis</em></td>
<td>4,753</td>
<td>232</td>
</tr>
<tr>
<td>CdrA</td>
<td><em>P. aeruginosa</em></td>
<td>2,154</td>
<td>129</td>
</tr>
<tr>
<td>FHA</td>
<td><em>B. pertussis</em></td>
<td>3,590</td>
<td>128</td>
</tr>
<tr>
<td>BcpA</td>
<td><em>Burkholderia thailandensis</em></td>
<td>3,147</td>
<td>233</td>
</tr>
</tbody>
</table>
phases in biofilm maturation and stabilization (86, 89). It is worth noting that LapA is not present in pathogenic pseudomonads, such as *P. aeruginosa* and *Pseudomonas syringae* (85, 90), suggesting that while LapA is crucial for biofilm formation in environmental pseudomonads, pathogenic strains have developed other mechanisms for adhesion. *P. aeruginosa* strains have a *lapF* gene, whereas no LapF ortholog has been found in *P. syringae* (90). A similar observation is reported for Bap in *S. aureus*, where *bap* genes are absent from the genomes of human pathogenic strains (91).

Once secreted via the T1SS, all Baps remain loosely anchored to the outer membrane and are frequently released into the extracellular space. The nature of the interaction between the cell envelope and Baps remains unknown, but various hypotheses have been proposed: (i) Baps could transiently interact with the T1SS apparatus upon secretion; (ii) Baps could interact with an unknown surface-exposed outer membrane protein; or (iii) released Baps could eventually form in homotypic interaction with the residual surface-associated Baps (78, 92). Recent studies of the large adhesion SiiE from *Salmonella enterica* revealed that the retention of SiiE on the cell surface is modulated by pH, ionic strength, and osmolarity and is probably due to the interaction of the SiiE coiled-coiled domains with the proteins forming the T1SS pore (93). In addition, extensive studies of the Baps LapA and LapF from *P. putida* show that these proteins are important components of the biofilm extracellular matrix and could be a link between the cells and matrix exopolysaccharides (94–96). During biofilm dispersal, LapA is cleaved from the cell surface by the specific protease LapG and released into the extracellular medium, freeing the bacteria from the biofilm matrix (94). Thus, the balance between retention on the cell surface and the release of Baps into the extracellular space may play a role in the regulation of cell adhesion to surfaces and eventually biofilm formation and dispersion.

**T5SS Autotransporter Adhesins**

The other major class of nonfimbrial adhesins is secreted by the T5SS. One of the largest groups of secreted proteins in Gram-negative bacteria, T5SS proteins display a two-step process: proteins are first transported from the cytoplasm to the periplasm via the Sec machinery and are then secreted across the outer membrane through a channel formed by a β-barrel pore (Fig. 3) (97). Originally thought to be self-sufficient for proper assembly on the cell surface, this type of protein was called “autotransporter.” However, more recent studies suggest that the Bam complex, the machinery responsible for proper folding and proper insertion of outer membrane proteins into the outer membrane, is required for proper assembly of T5SS (98, 99).

All T5SS proteins share common structural and functional features (Fig. 4): (i) an N-terminal Sec-dependent signal peptide, (ii) a passenger domain providing the protein function, and (iii) a C-terminal β-barrel domain that allows secretion of the passenger domain. Once the protein is processed, the β-barrel domain integrates into the outer membrane to form a pore through which the passenger domain is secreted to the cell surface (for recent reviews, see references 79, 100).

The T5SS autotransporter family is represented by three distinct groups: (i) the monomeric autotransporters, (ii) the trimeric autotransporters, and (iii) the two-partner secretion systems.

**Monomeric autotransporter adhesins (MAAs)**

This group is the largest group of T5SS proteins. In monomeric autotransporters, passenger and secretion domains are integrated into a single multidomain protein (Fig. 3). The secretion β-barrel domain (250 to 300 amino acids) has high sequence similarity among monomeric autotransporters and is predicted to form a pore comprised of 14 antiparallel β-strands (97). Conversely, the passenger regions consist of repetitive amino acid motifs, highly variable in sequence and size, responsible for the difference in specificity of each protein (101). The properties of autotransporters are very diverse, because they can be virulence factors, toxins, or adhesins, among other functions. The majority of the studies of these autotransporter adhesins focus on specific interactions and host cell-bacterial adhesion, and only a few monomeric autotransporter adhesins have been shown to play a role in biofilm formation and nonspecific adhesion to abiotic surfaces (Table 3).

One of the most studied monomeric autotransporters is Ag43 from *E. coli*. Based on sequence similarity with Ag43, several other monomeric autotransporter adhesins have been identified in *E. coli*, and a few have been shown experimentally to be involved in cell aggregation and biofilm formation (Table 3) (102). Ag43 is present in the majority of commensal and pathogenic *E. coli* strains (103) and promotes autoaggregation and biofilm formation (104, 105). The exact role of monomeric autotransporters in biofilm formation has not been entirely elucidated yet, but recent experiments suggest that Ag43, like other related autotransporters including AIDA (adhesin involved in diffuse adherence), may mediate cell-cell aggregation by homotypic interactions that could eventually play a role in biofilm formation.
formation (106, 107). In addition, it has been shown that different monomeric autotransporter adhesins, like Ag43 and AIDA, can cross-interact, leading to the formation of mixed cell aggregates (79, 101, 107). It has been shown that many autotransporters are O-glycosylated proteins, like Ag43, AIDA, and TibA in E. coli (108–110). However, this glycosylation does not play a role in cell-cell aggregation or biofilm formation (109, 112). Ag43 expression is controlled by phase variation during biofilm formation (101). A recent study highlighted the importance of Ag43 phase variation during biofilm formation mediated by both Ag43 and other adhesins in E. coli (113). Indeed, Ag43+ bacteria are physically selected for in the biofilm, probably due to their inherent ability to auto-aggregate, which may subsequently modulate interaction with surfaces via the production of other adhesins (113). The recent resolution of the crystal structure of Ag43 reveals a “Velcro-like” organization similar to the self-oligomerization of the Hap autotransporter from H. influenzae (114), mediating Ag43-Ag43 interactions and probably Ag43 interactions with other autotransporters and promoting cell-cell aggregation (115).

Trimeric autotransporter adhesions (TAAs)
All the members of the TAA family described so far are nonfimbrial adhesin proteins (92). Numerous TAAs have been reported to specifically bind to biotic surfaces on host cells, or host extracellular matrix components including fibronectin or collagen, and only a few studies have focused on the role of these adhesins in biofilm formation and attachment to abiotic surfaces (Table 3).

While the overall organization of these autotransporters is similar to the monomeric autotransporters, TAAs usually have a shorter C-terminal secretion domain (50 to 100 amino acids) with four β-strands. The formation of a full-size 12-β-strand pore is achieved by trimerization (116). The passenger domain contains conserved elements designated as head, connector, and stalk domains (117). The head harbors the adhesive properties, while the connector and stalk primarily function to extend the head domain away from the bacterial cell body (117). Unlike the monomeric autotransporters described above, TAA are not cleaved or released into the extracellular space (106).

The most studied TAA in the multifunctional nonfimbrial adhesins is YadA from Yersinia spp. (118). This adhesin has been linked to virulence, adhesion to host cells, and autoaggregation (118). However, the putative role of YadA in adhesion to abiotic surfaces and biofilm formation is poorly understood. A specific 31-amino acid motif, present only in the N-terminal sequence of YadA from Yersinia pseudotuberculosis, is required for cell aggregation and biofilm formation, but the mechanism of adhesion is unknown (119). Only a few other TAAs have been reported to play a role in biofilm formation (Table 3). UpaG is involved in cell-cell interactions, promoting biofilm formation to abiotic surfaces in E. coli (120). SadA, an ortholog of UpaG in S. enterica and S. typhimurium, has been shown to mediate cell aggregation and biofilm formation in cells unable to express long O-antigen on the cell surface (121), suggesting that bacteria need to be in close proximity for SadA to mediate cell-cell interactions. Recently, BbfA (Burkholderia biofilm factor A) has been shown to play a major role in biofilm formation in Burkholderia pseudomallei, probably by mediating cell aggregation (122).

Two-partners secretion system (TPS)
TPS proteins form another group of autotransporters utilizing T5SS. In this family, the secretion mechanism is very similar to the ones described above, but the passenger and membrane anchor domains are encoded by two distinct genes, usually organized in a single operon. One gene encodes the secretion signal and the adhesin domain, and the other encodes the outer membrane β-barrel pore (79) (Fig. 3). The pores are highly conserved proteins of typically 500 to 800 amino acids (123). The effectors are large proteins and can be highly variable, but they all contain two common features: (i) a β-helix or β-solenoid secondary structure and (ii) a conserved N-terminal domain, the TPS domain (123). The TPS domain provides specific recognition of the appropriate pore for translocation through the outer membrane (116).

Filamentous hemagglutinin proteins are typical adhesins exported by the TPS (Table 3) and are usually involved in tight interaction between the bacterium and a specific receptor on the host cell. Their involvement in biofilm formation and nonspecific adhesion to abiotic surfaces has been studied only sparsely. Filamentous hemagglutinins have been shown to play a role in biofilm formation by different plant pathogens, including X. fastidiosa or various Xanthomonas spp. (124–126). Unlike the two other types of autotransporter adhesins described above, it seems that filamentous hemagglutinins from these species do not mediate cell-cell interactions and autoaggregation, but rather attach cells to abiotic surfaces, thereby facilitating their interaction with each other (125). Filamentous hemagglutinins also...
play a role in biofilm formation in the human pathogens *H. influenzae* (127) and *Bordetella pertussis* (128). Filamentous hemagglutinin from *B. pertussis* is a critical virulence factor and is involved in interactions between bacterial cells, as well as interactions between the bacteria and abiotic surfaces (128). In addition, it has been shown that the hemagglutinins HMW (high molecular weight protein) 1 and HMW 2 are found around *H. influenzae* cells grown in a biofilm, as well as interacting with the biofilm extracellular matrix (127), strongly suggesting that these proteins play an important role in adhesion to abiotic surfaces by linking the bacteria to the biofilm matrix and stabilizing the overall architecture.

Another example of a nonfimbrial adhesin belonging to the TPS group is CdrA (c-di-GMP regulated TPS A) in *P. aeruginosa*. This adhesin is transported by CdrB (c-di-GMP regulated TPS B) and is expressed when the level of c-di-GMP in the cell is high or in biofilm cultures (129). Once secreted outside the cell, CdrA is proposed to bind to the exopolysaccharide Psl to promote cell aggregation and biofilm formation. This adhesin is part of the biofilm matrix and plays a role in maintaining biofilm structural integrity, probably by bundling individual Psl strands together and/or by anchoring Psl fibers to the bacteria in the biofilm (129).

**POLYSACCHARIDE ADHESINS**

In addition to the various protein adhesins described above, many bacterial species produce extracellular polysaccharides that promote adhesion. These polysaccharides can be firmly associated with the cell surface, forming a capsule (capsular polysaccharide), or be loosely associated or even released from the cell surface (extracellular polysaccharide [EPS]). The difference between capsular polysaccharide and EPS is usually experimentally defined and may have limited physiological relevance. From an adhesive point of view, it is more appropriate to distinguish protective from aggregative polysaccharides. While protective polysaccharides provide a protective barrier around the cell, the aggregative polysaccharides have adhesive and/or cohesive properties that are exploited by bacteria to mediate adherence to surfaces and/or reinforce structural integrity of the biofilm (130, 131). Although protective polysaccharides could also contribute to the adhesive properties of bacterial cells, only the aggregative polysaccharides that serve as adhesins will be discussed in this section. For simplicity, aggregative polysaccharides will be referred to as EPS.

**Structure and Function**

EPSs often have long chains (molecular weight > 10^6 Da) composed of a repetition of the same sugar residue (homopolysaccharide) or a mixture of neutral and/or charged sugar residues (heteropolysaccharide). These polymers are mostly negatively charged, but neutral and even positively charged EPSs also exist (6, 132–134). The adhesiveness of EPSs strongly depends on chain conformation and is greatly influenced by substituents that modify interchain and intrachain interactions (135). The polar and hydrogen bonding functional groups of polysaccharides, such as ethers and hydroxyls, provide good adhesion to polar surfaces. The adhesiveness of EPSs can be strongly influenced by the presence of divalent cations such as Ca^{2+} and Mg^{2+} (6, 132–134, 136).

The degree of acetylation of a polysaccharide can drastically modify its cohesiveness and adhesiveness (137–141). Partial deacetylation of the well-characterized poly-β-1,6-N-acetyl-d-glucosamine EPS (PGA) is required to initiate biofilm formation by *E. coli*, *S. aureus*, and *Staphylococcus epidermidis* (143–145). Similarly, the selective O-acetylation of mannuronate residues on alginate, a polysaccharide secreted by *P. aeruginosa*, seems to improve biofilm adhesion and increase adhesion to host surfaces (137, 139, 146). Furthermore, partial deacetylation of *E. coli* PGA is required to facilitate the secretion of the polymer through the outer membrane (143). On the other hand, partial deacetylation of holdfast in *C. crescentus* and *Asticcacaulis biprosthecum* appears to be essential to maintain the structure, the adhesiveness, and the retention of the polymer on the cell surface in both species (141). In this case, the deacetylation of polysaccharides might facilitate the conformational transition of the polymer chain from random coils to ordered helices, promoting better interchain interactions and more stiffness of the polymer (138).

**Biosynthesis, Secretion, and Anchoring**

Although chemically diverse, the molecular mechanisms by which EPSs are assembled and exported to the cell surface can be categorized into three distinct mechanisms: (i) the Wzx/Wzy transporter-dependent pathway, (ii) the ABC transporter–dependent pathway, and (iii) the synthase-dependent pathway (Fig. 5). These three mechanisms have been extensively reviewed, and only a brief overview will be presented in this section (for more detail see reference 131).

In the Wzx/Wzy-dependent pathway, each polysaccharide repeat unit is first assembled on an undecaprenyl
phosphate acceptor moiety in the cytoplasm, which is then transported across the inner membrane before being polymerized into a high molecular weight polysaccharide in the periplasm. In contrast, the entire polysaccharide chain is assembled in the cytoplasm on a lipid acceptor in the ABC transporter–dependent pathway. Although mechanistically different, these two pathways use a similar mechanism to facilitate EPS export across the periplasm and through the outer membrane. Both pathways use a large protein complex spanning the periplasmic space, formed by an outer membrane polysaccharide export pore and a polysaccharide co-polymerase. In the synthase-dependent pathway, both the polymerization and the transport of the polymer across the inner membrane are carried out by the same membrane-embedded glycosyl transferase (131, 147). The polymer is protected by a molecular chaperone, a tetratricopeptide repeat–containing protein, and then exported across the outer membrane through a β-barrel porin (147).

When associated with the cell surface, the exact connection between EPS and the outer membrane is not always known and usually involves a linker, which can be a sugar, lipid, or protein. For EPS exported through

**FIGURE 5** Polysaccharide biosynthesis pathways. Overview of the Wzx/Wzy–, ABC-transporter–, and synthase–dependent exopolysaccharide biosynthesis pathways. Only the key components for each pathway are indicated on the diagram. In the Wzx/Wzy–dependent pathway, the polysaccharide repeat unit assembly is initiated on an undecaprenyl phosphate acceptor moiety located in the inner leaflet of the inner membrane, which is then transported across the inner membrane by the flippase, Wzx. The polymerization into high–molecular weight polysaccharide occurs in the periplasm by the action of the polymerase Wzy. The export and secretion of the polysaccharide through the outer membrane are facilitated by the outer membrane polysaccharide export (OPX) and the polysaccharide copolymerase (PCP) protein families. Depending on the polysaccharide being synthesized, the nascent polymer could be anchored to the outer membrane via a specific protein, such as Wzi. In the ABC transporter–dependent pathway, the entire polysaccharide chain is assembled into the cytoplasm on a lipid acceptor that is then transported across the inner membrane by the ABC transporter. As observed for the Wzx/Wzy–dependant pathway, the export and secretion of the polysaccharide through the outer membrane also involve the OPX and PCP protein families. In the synthase–dependent pathway, both the polymerization and the transport of the polymer across the inner membrane are carried out by the same membrane-embedded glycosyl transferase. The export and secretion of the polysaccharide through the outer membrane are facilitated by a molecular chaperone and a β-barrel porin. Abbreviations: IM, inner membrane; CW, cell wall; OM, outer membrane. doi:10.1128/Microbiolspec.MB-0018-2015.f5
the ABC transporter–dependent pathway, the conserved phospholipid terminus is attached at the reducing end of the polysaccharide via a poly-3-deoxy-D-manno-oct-2-ulosonic acid (also known as KDO) linker that is responsible for proper translocation and contributes to the attachment of the polymer to the cell surface (148), although ionic interactions between the core region of the lipopolysaccharide (LPS) and the polymer may also be involved (159). As observed for LPS O-antigen, carbohydrate chains forming the capsular or K-antigen (derived from the German word *Kapsel* [150]) may also be linked to the lipid A core of an LPS molecule, but the distinction from a traditional O-antigen remains subtle and purely operational (151, 152). Finally, polysaccharides can be anchored to the cell surface via dedicated proteins (153–155). In *E. coli* and *K. pneumoniae*, the outer membrane protein Wzi is a critical factor in the anchoring of the K30 type I capsule to the cell surface (153, 155). The 2.6Å resolution structure of Wzi suggests that Wzi may act as a lectin binding to the nascent polymer once translocated through the Wza translocase, but other cell surface components may also play a role (153). In *C. crescentus*, the HfaA, HfaB, and HfaD proteins provide an extremely strong anchor for the holdfast polysaccharide to the cell envelope, as discussed below (154). Alternatively, polysaccharides can be anchored to the cell surface by glycosylation of proteins, a system primarily utilized to decorate flagellar proteins and protein adhesins (for review see references 156, 157).

### Polysaccharide Diversity

The composition and the structure of EPS can vary both between and within species (148), and it is now well accepted that bacteria are able to produce different types of EPS to provide adhesive adaptability under varying conditions (Table 4). Most of these carbohydrate polymers generate a highly hydrated and extensive layer surrounding the cell, but a few bacterial species adhere to surfaces by using a clearly defined, discrete patch of polysaccharide (158–160). We will focus on the discrete aggregative polysaccharides in this section.

There are diverse instances of discrete adhesive polysaccharide mediating surface attachment. Among these is the well-described unipolar attachment to surfaces of *Alphaproteobacteria*, such as *C. crescentus* and *A. tumefaciens*, and the less common discrete slime deposition by the motile *Deltaproteobacterium, Myxococcus xanthus* (Fig. 6). In both cases, the discrete polysaccharide provides a specific function for different phases of the life cycle, supporting a transient or an irreversible attachment with the surface. In this section, the unipolar polysaccharide adhesin produced by *C. crescentus* and by some *Rhizobiales* and the adhesive slime produced by *M. xanthus* will be discussed.

### Holdfast

*Caulobacter* spp. and other stalked *Alphaproteobacteria* (161) synthesize a polar polysaccharide called holdfast (162) (Fig. 6). Only a small amount of holdfast is produced at a discrete site on the cell surface and can be

<table>
<thead>
<tr>
<th>Polysaccharide</th>
<th>Organism</th>
<th>Composition/structure</th>
<th>Reference</th>
</tr>
</thead>
<tbody>
<tr>
<td>Alginate</td>
<td><em>P. aeruginosa</em></td>
<td>β-1,4-linked mannuronic acids and guluronic acids</td>
<td>234, 235–239</td>
</tr>
<tr>
<td>Cellulose</td>
<td><em>Glucanacetobacter xylinus, A. tumefaciens, Rhizobium leguminosarum</em> bv. <em>Trifolii, Sarcina ventriculi, Salmonella spp., E. coli, K. pneumoniae</em></td>
<td>β-1,4-linked β-D-glucose</td>
<td></td>
</tr>
<tr>
<td>Holdfast</td>
<td><em>Caulobacter spp., Asticcacaulis biprosthecum, Hyphomonas adherens, Hyphomonas rosenbergii, Hyphomicrobiurn zavarzinii, Mariculis maris, Oceanicaulis alexandri</em></td>
<td>Suspected to contain β-1,4-linked N-acetyl-α-glucosamine, but the exact composition and structure remain unknown</td>
<td>160, 163, 166, 240–243</td>
</tr>
<tr>
<td>PGA</td>
<td><em>E. coli, Yersinia pestis, Bordetella spp., Actinobacillus spp., P. fluorescens</em></td>
<td>β-1,6-linked N-acetyl-α-glucosamine</td>
<td>244, 245</td>
</tr>
<tr>
<td>Psl</td>
<td><em>P. aeruginosa</em></td>
<td>Repeating pentasaccharide of 3 mannose, 1 rhamnose, and 1 glucose</td>
<td>246–248</td>
</tr>
<tr>
<td>Pel</td>
<td><em>P. aeruginosa, P. fluorescens</em></td>
<td>Unknown, but reported to be a glucose-rich polysaccharide polymer</td>
<td>246, 249, 250</td>
</tr>
<tr>
<td>Slime</td>
<td><em>M. xanthus</em></td>
<td>Suspected to contain α-D-mannose or α-D-glucose residues, but the exact composition and structure remain unknown</td>
<td>192</td>
</tr>
<tr>
<td>UPP</td>
<td><em>A. tumefaciens</em></td>
<td>Suspected to contain N-acetyl-α-D-glucosamine residues, but the exact composition and structure remain unknown</td>
<td>68, 179</td>
</tr>
</tbody>
</table>
FIGURE 6 Selected examples of discrete polysaccharides. AF488-conjugated wheat germ agglutinin lectin labelling of the holdfast in (A) *C. crescentus*, (B) *A. biprosthecum* (courtesy of Chao Jiang), (C) *Asticcacaulis excentricus* (courtesy of Chao Jiang), and (D) *Hyphomicrobium vulgare* (courtesy of Ellen Quardokus). (E) AF488-conjugated wheat germ agglutinin lectin labelling of the UPP in *A. tumefaciens*. (F) FITC-conjugated ConA lectin labelling of the slime in *M. xanthus*. doi:10.1128/microbiolspec.MB-0018-2015.f6
observed either at one of the cell poles or at the tip of a thin cylindrical extension of the cell envelope referred to as the stalk. The holdfast is responsible for permanent adhesion to a surface and can maintain attachment for many generations, presumably to provide better access to limited nutrients under flow (163). The holdfast is also crucial for biofilm formation (162). *C. crescentus* holdfast secretion is regulated both developmentally and by contact dependence (68). *C. crescentus* exhibits a dimorphic life cycle that begins as a motile swarmer cell. Unless they come in contact with a surface, swarmer cells are able to swim for a period equivalent to one-third of the cell cycle, after which they differentiate into stalked cells. During this developmentally programmed transition, swarmer cells release their flagellum, retract their pili, and synthesize a holdfast and a stalk at the same pole. The small protein regulator HfA is crucial for holdfast production at the correct time during the cell cycle, as well as under the correct nutritional conditions (164). In addition to the developmental timing of holdfast secretion, if a swarmer cell encounters the surface, the coordinated action of the flagellum and pili stimulates early holdfast secretion, thereby driving the rapid, just-in-time transition from reversible to irreversible attachment (68). The addition of crowding agents that impede the rotation of the flagellum also stimulates the production of holdfast, suggesting that increased load on the flagellum triggers holdfast synthesis (68).

Two main gene clusters in *C. crescentus* have been identified that are required for holdfast synthesis (hfs) and holdfast anchoring to the tip of the stalk (hfa). The hfs cluster encodes proteins involved in the biosynthesis and secretion of the polysaccharide in a process similar to the Wzx/Wzy transporter-dependent pathway (160, 165). The hfa cluster encodes proteins crucial for attaching the holdfast to the cell envelope. Mutations in the hfa locus result in inefficient attachment of the holdfast to the tip of the stalk, causing holdfast shedding into the culture supernatant (154). The hfa locus is a single operon comprising hfaA, hfaB, and hfaD. The HfaA, HfaB, and HfaD proteins are associated with the outer membrane and are polarly localized. HfaA and HfaD form high molecular weight complexes that share properties with amyloid proteins, and HfaB plays a role in the secretion of both proteins (154). Although the exact anchoring mechanism between the polysaccharide and the high molecular weight complexes formed by HfaA and HfaD remains unknown, the HfaA, HfaB, and HfaD proteins provide an extremely strong anchor for the holdfast polysaccharide to the cell envelope of *C. crescentus* (154).

Holdfast is bound specifically by wheat germ agglutinin, a lectin that specifically recognizes N-acetyl-D-glucosamine (GlcNAc)–containing polymers. In addition, holdfast is sensitive to lysozyme, a glycolytic enzyme specific for cleavage of β-1,4 linkages in oligomers of GlcNAc, suggesting that the holdfast is mostly comprised of β-1,4-linked GlcNAc (163, 166). The exact composition and structure of the holdfast is still unknown, due in large part to the small quantity synthesized by cells and its insoluble nature. The holdfast of *C. crescentus* has a gel-like nature and exhibits an impressive adhesive force in the µNewton range, the equivalent of 10,000 psi, making holdfast one of the strongest known biological adhesives characterized (166, 167). GlcNAc polymers have been proposed to provide elastic properties to the holdfast (166). Recent force spectroscopy experiments suggest that additional adhesive components within the holdfast might be responsible for the bulk of adhesion (168). The measure of time-dependence of holdfast rupture force on a variety of substrates suggests the existence of discrete and cooperative events of single adhesin molecules that may act in concert with the GlcNAc polymers (168). As mentioned earlier, the degree of deacetylation strongly influences the physical properties of the holdfast polysaccharide (141). The deletion of hfsH, a polysaccharide deacetylase, strongly affects the adhesiveness and the cohesive properties of holdfast (141).

Although *C. crescentus* holdfast remains the best studied, other stalked bacteria including *Hypomonas*, *Hyphomicrobium*, *Asticcaulis*, other freshwater *Caulobacter*, and a variety of marine bacteria also produce a discrete holdfast polysaccharide (Fig. 6) (for a review see reference 169). These polysaccharides localize to the pole of the cell and provide strong adhesion to surfaces in marine and freshwater environments. The holdfast biosynthesis gene clusters of the other species often contain additional genes for gycosyltransferases or polysaccharide modification enzymes as compared to *C. crescentus* (169), suggesting that holdfast composition may vary depending on the environment in which the different species attach.

**Polar surface polysaccharide from Rhizobiales**

In addition to the *Caulobacteriales* holdfast, other members of the *Alphaproteobacteria* class exhibit similar polar polysaccharides that are involved in adhesion. The *Rhizobiales* *A. tumefaciens*, *Rhizobium leguminosarum*, and *Bradyrhizobium japonicum*, can form a pathogenic or endosymbiotic association with certain plants (170). This association starts with the attachment of
the bacteria to the host plant tissues, a multistep process mediated by bacteria-secreted polysaccharides, bacteria-secreted adhesins, or in some cases, by plant-produced lectins (170–173). Once in contact with the plant surface, bacteria may produce cellulose fibrils to form a stable biofilm around the tips of root hairs or the plant tissues (174). Several different surface polysaccharides, including LPS and capsular acidic polysaccharides have been shown to play a role in plant infection and colonization. In addition, A. tumefaciens, R. leguminosarum, and B. japonicum produce a unipolar polysaccharide that appears to be essential for the initial step of adhesion (171).

A. tumefaciens forms complex biofilms on abiotic surfaces and plant roots that eventually cause “crown gall” disease. Unlike R. leguminosarum and B. japonicum, A. tumefaciens is pathogenic and does not benefit the plant (175). The infection involves first the attachment of the bacteria to the plant surface and then the transfer of a DNA segment carried on the tumor-inducing (Ti) plasmid into the host plant genome. It has been shown that A. tumefaciens produces several different EPSs: succinoglycan, cellulose, β1-2 glucans, and β1-3 glucans. However, more recent studies indicate that the initial attachment to the plant root surface and abiotic surfaces is mediated by a coordinated action of flagella and the unipolar polysaccharide (UPP) (68, 178, 179). UPP is crucial for permanent attachment, but by comparison with the holdfast of C. crescentus, UPP is only observed upon surface contact (68). More recently, it has been proposed that secretion of UPP is stimulated by an increase of the intracellular level of c-di-GMP, suggesting that surface contact may stimulate the increase of c-di-GMP through the activity of diguanylate cyclases or phosphodiesterases by an as yet unknown mechanism (179).

The secretion of UPP is abolished by deletion of the uppABCDEF locus (178, 179). The genes within the uppABCDEF locus share similarity with several genes within the hfs locus in C. crescentus, most notably UppC with HfsD, the predicted Wza outer membrane export porin (175). No homologues of the hfa genes, which are involved in holdfast anchoring in C. crescentus, have been identified in agrobacterial genome sequences (175), suggesting that UPP and holdfast are anchored to the cell surface via different mechanisms. As observed for holdfast, UPP reacts specifically with wheat germ agglutinin, suggesting that the UPP also contains GlcNAc residues, but its exact composition and structure remain unknown (68, 179).

R. leguminosarum forms an endosymbiotic association with certain leguminous plants such as Trifolium (clover), Pisum (pea), Vicia (vetch), and Phaseolus (bean) to potentially nodulate and differentiate into nitrogen fixing bacteroids (180). This bacterium also synthesizes a discrete polar polysaccharide that is responsible for specific adhesion to plant receptors. The primary attachment of R. leguminosarum to host plant root hairs involves bacteria-secreted polysaccharides and plant-produced lectins (171, 172). The plant-produced lectins, such as the pea (P. sativum) lectin and the vetch (V. sativa) lectin are hypothesized to recognize and bind to a polarly localized glucomannan exopolysaccharide present at the surface of R. leguminosarum, promoting bacterial attachment at one pole (171–173). The glucomannan exopolysaccharide is mainly composed of mannose and glucose with minor amounts of galactose and rhamnose (171). Terminal mannose and glucose were identified, in addition to 1,2-mannose, 1,4-glucose, and 1,3,4-linked glucose, but the exact structure remains unknown (171). At least one gene (gmsA), whose deletion abolishes glucomannan secretion and biofilm formation on root hairs, has been identified as being involved in glucomannan biosynthesis (174). Interestingly, gmsA belongs to a conserved gene locus among other rhizobia and, more specifically, A. tumefaciens. This locus shares sequence similarity with the uppABCDEF locus in A. tumefaciens, suggesting that glucomannan could be biosynthesized through a similar pathway as UPP (178). Glucomannan is required for initial attachment only under moderate acidic conditions. Under neutral or alkaline conditions, attachment to root hairs is mediated by Rhexadhesin, a calcium-binding protein, or rhizobium-adhering proteins, named “Rap” (171, 173, 181).

B. japonicum forms an endosymbiotic association with soybean plants. It was proposed that initial attachment was mediated by the plant lectin SBA (soybean agglutinin), but another carbohydrate-binding protein, called BJ38, has been subsequently identified (176). Both SBA and BJ38 bind to a polarly localized surface polysaccharide produced by B. japonicum, but surprisingly, SBA and BJ38 recognize polysaccharides at opposite ends of the cell (176). It was suggested that the pole bound by BJ38 is probably involved in cell-cell and cell-host attachment since BJ38 labeled the point of bacterial attachment (176, 182). However, the exact nature of this interaction and the composition of this polar polysaccharide remain to be determined (176, 182). More recently, SBA has been shown to promote biofilm formation even in the absence of plant roots, suggesting that SBA also contributes to adhesion on abiotic surfaces (183).
In conclusion, the use of a polar adhesin appears to be a common feature shared by some Alphaproteobacteria to mediate adhesion of the bacterial cell to biotic or abiotic surfaces. The conservation of this feature suggests that the resulting asymmetric adhesion is important for the fitness of these species. However, future studies will be needed to identify the adhesive mechanism behind these strongly adhesive unipolar polysaccharides, their regulation, and their biosynthesis.

**Slime**

By comparison with the discrete unipolar polysaccharide of *C. crescentus*, the deltaproteobacterium *M. xanthus* exhibits an unusual patchy localization of an adhesive polysaccharide referred to as slime (Fig. 6). *M. xanthus* moves across solid surfaces by both twitching and gliding motility. While twitching motility results from polar retractile type IV pili, gliding motility results from the action of internal molecular motors that exert traction against the substrate through a large envelope-spanning complex (184–187). Under low-nutrient conditions, *M. xanthus* cells coordinate their motility to merge into multicellular structures called fruiting bodies, where cells follow a developmental program that eventually triggers their differentiation into stress-resistant spores. Under high-nutrient conditions, spores germinate and form predatory swarms that secrete degradative enzymes to lyse prey bacteria (188). While moving over surfaces, slime is observed in the wake of motile bacteria.

Slime has been observed for more than three decades (189) and has been proposed to be directly responsible for gliding motility (190), but recent evidence suggests the slime most likely promotes adhesion of the gliding motors to the substrate by a “paste-release” mechanism (191).

When observed at high contrast by a noninvasive technique called Wet-SEEC (wet-surface enhanced ellipsometry contrast microscopy) COMP, slime trails appear to be secreted inconsistently behind the cell as extended continuous segments separated by patchy and localized deposits (191, 192). On cells, slime is unevenly distributed, forming conspicuous patches distributed at regular intervals on the surface of the cell body (192). Careful examination suggests that slime is locally concentrated and deposited underneath the cell body at fixed and discrete positions that colocalize with the gliding machinery. It has been suggested that the gliding machinery itself may produce its own adhesion substrate, enhancing *Myxococcus* substrate adhesion and allowing cell gliding on a larger range of substrates, but the exact anchoring mechanism between the polymer and the tip of the machinery remains to be elucidated (192).

Although slime reacts with Concanavalin-A, a lectin specific to internal and nonreducing terminal α-D-mannose or α-D-glucose, its exact composition remains unknown (192). Moreover, slime secretion remains unaffected by the deletion of genes involved in the secretion of exopolysaccharides through the Wzx/Wzy transporter-dependent pathway or in the biosynthesis of the O-antigen, suggesting that slime is secreted by an alternative secretion pathway. More recently, slime has been shown to contain embedded outer membrane vesicles, which might be involved in cell-cell communication or cell-cell transfer of outer membrane proteins between *Myxococcus* cells (191). Slime may play multiple roles by not only providing specific adhesion for the gliding machinery, but also by potentially supporting a form of cell-cell communication between distant *Myxococcus* cells sharing the same track.

**CONCLUSION**

During the last decade a large repertoire of bacterial adhesins has been identified. The recent advances in our understanding of the secretion, assembly, and regulation of these bacterial adhesins demonstrate that, even if they share some key structural and regulatory factors that determine their expression at the surface of cells, their level of specificity varies significantly depending on the targeted surface. This characteristic clearly illustrates the ability of bacteria to successfully adapt and adhere to virtually all natural and man-made materials. This article reviewed major protein and localized discrete polysaccharide adhesins, but the large variety of adhesins suggests that virtually any biological molecule made by the bacterium can be used to support or modulate its adhesion to surfaces. Even previously unsuspected biological molecules, such as extracellular DNA and lipids, have been shown to play a role in the process of adhesion or biofilm maturation (193, 194). Recent discoveries in the regulation and the modulation of adhesion suggest that this process is much more complex and dynamic than originally anticipated. We have just begun to understand the factors that govern the temporal regulation of the expression of adhesin molecules, but many questions concerning the nature of signals triggering the secretion of the proper adhesin or the interaction between the different types of adhesins remain unanswered. The recent advent of single-cell approaches will yield a better understanding of the detailed roles of each adhesive factor and the environment during this
transition from reversible to irreversible attachment (195). Surprisingly, even with decades of research, the exact molecular mechanisms that support or modulate the interaction between adhesins and surfaces have seldom been characterized. Even if we can grasp the huge versatility of bacterial adhesives, we are lacking the most important knowledge about how this adhesion occurs.

A better understanding of the adhesive properties of these adhesins holds promise for future methods to control bacterial surface attachment, promoting it when beneficial and eradicating it when detrimental. In addition, an improved knowledge of this field will allow us to use certain bacterial adhesins as models of biological adhesives, which could offer an alternative strategy to their synthetic counterparts in industrial and medical applications. Indeed, biological adhesives demonstrate impressive performance in their natural context: they enable attachment to a broad variety of surfaces in aqueous environments and share desirable properties, such as sustainability, biodegradability, and biocompatibility, resulting in a much-reduced impact on the environment.

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