Cell. Mol. Life Sci. 66 (2009) 613 – 635
1420-682X/09/040613-23
DOI 10.1007/s00018-008-8477-4

Cellular and Molecular Life Sciences

Review

Pili in Gram-negative and Gram-positive bacteria – structure, assembly and their role in disease

T. Proft[a,c,*] and E. N. Baker[b,c]

[a] School of Medical Sciences, Department of Molecular Medicine & Pathology, University of Auckland, Private Bag 92019, Auckland 1142 (New Zealand), Fax: +64-9-373-7492, e-mail: t.proft@auckland.ac.nz
[b] School of Biological Sciences, University of Auckland, Auckland (New Zealand)
[c] Maurice Wilkins Centre for Molecular Biodiscovery, University of Auckland (New Zealand)

Received 08 August 2008; received after revision 24 September 2008; accepted 01 October 2008
Online First 27 October 2008

Abstract. Many bacterial species possess long filamentous structures known as pili or fimbriae extending from their surfaces. Despite the diversity in pilus structure and biogenesis, pili in Gram-negative bacteria are typically formed by non-covalent homopolymerization of major pilus subunit proteins (pilins), which generates the pilus shaft. Additional pilins may be added to the fiber and often function as host cell adhesins. Some pili are also involved in biofilm formation, phage transduction, DNA uptake and a special form of bacterial cell movement, known as 'twitching motility'. In contrast, the more recently discovered pili in Gram-positive bacteria are formed by covalent polymerization of pilin subunits in a process that requires a dedicated sortase enzyme. Minor pilins are added to the fiber and play a major role in host cell colonization.
This review gives an overview of the structure, assembly and function of the best-characterized pili of both Gram-negative and Gram-positive bacteria.

Keywords. Pili, fimbriae, pilin, cytoadherence, biofilms, lectin, twitching motility, sortase.

Introduction

Pathogenic bacteria have to attach to specific host cells as a crucial step in establishing an infection. This process is necessary for colonization of host tissue and is mediated by surface-exposed adhesins, which generally behave as lectins, recognizing oligosaccharide residues of glycoprotein or glycolipid receptors on the host cell. The presence of specific adhesion factors on the bacterial cell surface also determines the tropism of the pathogen to the tissues expressing certain surface receptors. In addition to the recognition of specific receptors, adhesins often also bind to structural elements of the basement membrane, such as collagen, fibronectin, etc. A general problem the bacterium faces is the net repulsive force caused by the negative charges of both bacterium and host cell. This can be overcome by a cell surface structure in which the adhesin is located at the tip of hair-like, peritrichous, non-flagellar, filamentous surface appendages known as pili (latin for 'hair') or fimbriae (latin for 'thread' or 'fiber'). The main structure, also known as the 'fimbrial rod' or 'pilus shaft', is composed of several hundred (probably thousands) of small 15 – 25 kDa subunits or pilins.
Pili are important virulence factors for several diseases, in particular infections of the urinary, genital and gastrointestinal tracts. Pili are also regarded as important targets for vaccine development. Although

* Corresponding author.

pili are often described as adhesive organelles, they have been implicated in other functions, such as phage binding, DNA transfer, biofilm formation, cell aggregation, host cell invasion and twitching motility. While pili in Gram-negative bacteria have been studied extensively over several decades, pili in Gram-positive bacteria have only recently been discovered.

Pili of Gram-negative bacteria

Pili of Gram-negative bacteria were first discovered in the late 1940s as receptors for bacteriophages [1]. Since then, these pili have been the focus of intensive research and much has been learned about their structure, assembly, post-translational modifications, regulation of expression and role in disease. This has been described in a number of excellent reviews and books. The pili of Gram-negative bacteria can be placed into four distinct groups based on their assembly pathways: a) pili assembled by the 'chaperone-usher pathway'; b) the Type IV pili; c) pili assembled by the extracellular nucleation/precipitation pathway (curli pili); and d) pili assembled by the 'alternative chaperone-usher pathway' (CS1 pilus family).

Chaperone-usher pathway assembled pili. The most extensively characterized pili of this group are the Type I pili, which are found throughout the family of *Enterobacteriaceae,* and the P pili from uropathogenic *Escherichia coli* (UPEC). The adhesive structures are heteropolymers composed of a small number of different protein subunits at various stoichiometries. A flexible fibrillar tip is joined end-to-end to a rigid rod and a single specific adhesive protein, which binds surface carbohydrates on host cells, is located at the distal end of the structure [2, 3]. Another group of adhesive structures assembled by the chaperone-usher pathway comprises the 'non-pilus adhesins'. Although originally characterized as afimbrial due to their amorphous or capsule-like morphology at low resolution, several members of this group can in fact be assembled into genuine fimbrial structures. The adhesive structures are generally homopolymers composed of a single protein subunit, like the Afa/Dr family of adhesins of *E. coli* [4, 5] and the polymeric F1 capsular antigen of *Yersinia pestis* [6, 7].

During pilus assembly, the pilus subunits (pilins) are secreted into the periplasmic space via the general secretory pathway and bind to a specific chaperone that assists in protein folding and prevents premature assembly of the subunits. The pilin/chaperone complex is then delivered to the outer membrane usher, which serves as a platform for pilus assembly. The usher protein forms a pore in the outer membrane that allows the passage of pilins, but not assembled pili (reviewed in [3, 8, 11]).

Type I pili. The structure of the Type I pilus was determined in 1969 by Brinton, who used electron microscopy, crystallography and X-ray diffraction [12]. A more recent electron microscopy study by Hahn et al. [13] showed that the adhesive organelle, encoded by the *fim* gene cluster (*fimA-fimH*), has a composite structure based on a 6.9 nm thick and 1–2 μm long helical rod which is formed by a right-handed helical array of 500–3000 copies of the main structural subunit FimA. The rod is connected via FimF to a short stubby 3 nm wide linear tip fibrillum containing FimG and the specific adhesin FimH [13, 14].

Type I pili are found on most *E. coli* strains and throughout the family of *Enterobacteriaceae*. They are the most prevalent type of pilus in UPEC, where they contribute significantly to bladder infections (cystitis) [15]. The bladder, usually a sterile site in healthy individuals, is the primary site of infection in more than 90% of all urinary tract infections (UTIs) [16]. Adhesion of UPEC to host cells within the urinary tract enables the bacteria to colonize and to avoid rapid clearance from the flow of urine. Specific binding to host cells is achieved by the specific FimH adhesin at the tip of the pilus structure. FimH has a two-domain fold with an N-terminal receptor-binding domain and a C-terminal pilin domain, both showing an immunoglobulin (Ig)-like fold [17] (Fig. 1A). However, the Ig-like fold of the pilin domain is incomplete, missing the seventh strand, which has to be provided either by the chaperone FimC or by another pilin subunit. FimH binds to mannose-containing receptors expressed by many types of host cells [18, 19]. Mannose groups are abundant on the glycan moieties of uroplakins, integral membrane glycoprotein receptors that coat the luminal surface of the bladder epithelium. FimH specifically binds to uroplakin UP1a, but not to the structurally related UP1b, which is glycosylated differently [18]. The basis for this specificity has recently been described by Xie et al. [20], who demonstrated that UPIa presents a high level of terminally exposed mannose residues that are capable of specifically interacting with FimH. In contrast, most terminally exposed glycans of UPIb are non-mannose residues.

Quantitative differences in the adhesiveness of Type I piliated *E. coli* has been attributed to FimH variants that differ in their receptor-binding domain [21]. All naturally occurring FimH variants bind tri-mannose receptors, but can vary in their affinity to bind mono-mannose receptors. FimH variants that bind mono-mannose groups with high affinity are preferentially

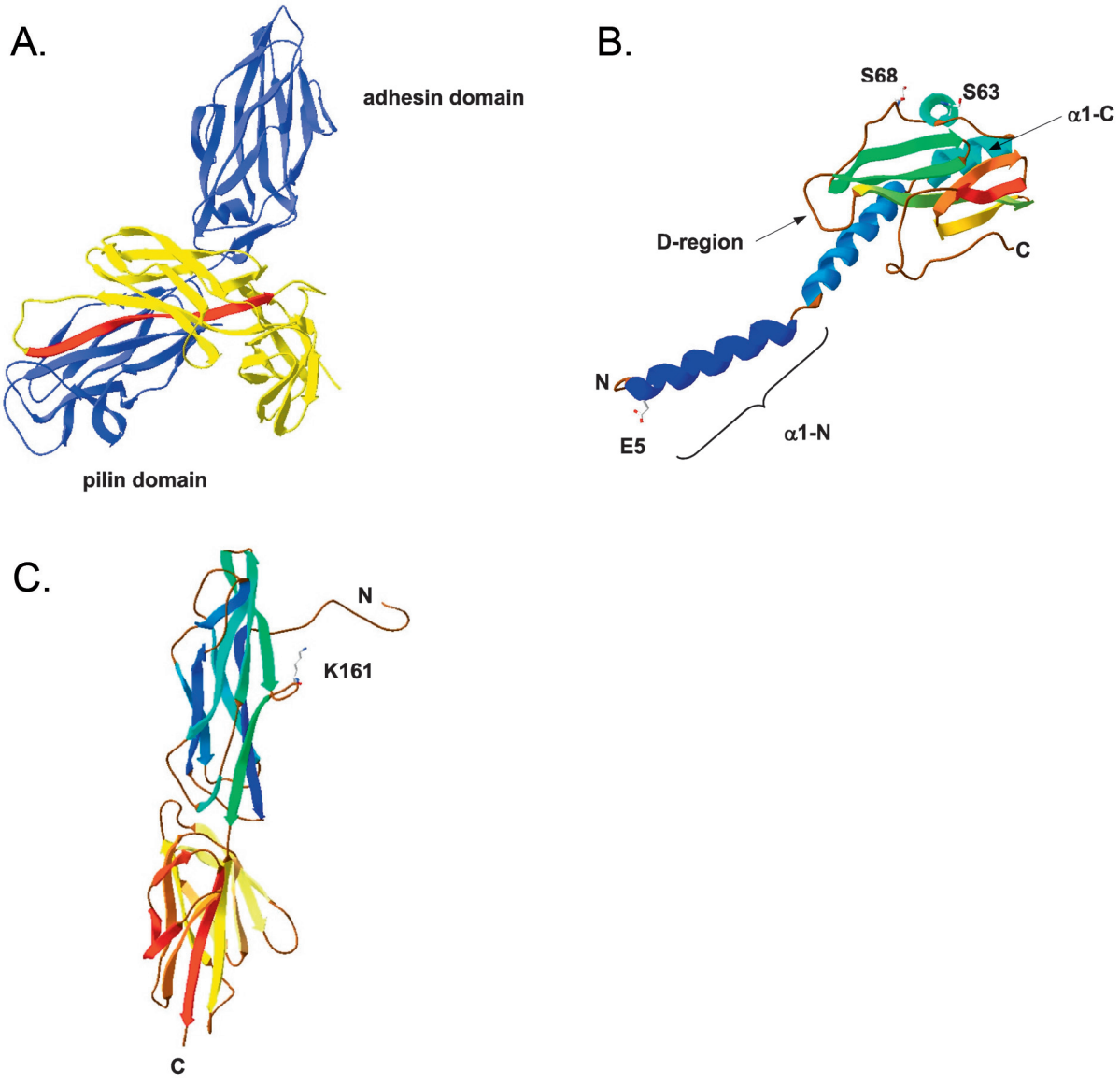

Figure 1. Protein structures of pilus component proteins from Gram-negative and Gram-positive bacteria. (*A*) Structure of the binary complex of the Type I pilus adhesin FimH (blue) and the chaperone FimC (yellow). FimH has a two-domain fold with an N-terminal receptor-binding domain and a C-terminal pilin domain. Both domains have an Ig-like fold, but this fold is incomplete in the pilin domain, and the missing seventh strand is completed by a donor strand from FimC (shown in red). (*B*) Protein structure of the *N. gonorrhoeae* GC major pilin. The N-terminal half of a long α-helix (α1-N) protrudes from a globular head (α1-C) giving the molecule a "ladle-like" shape. The interaction between the negatively charged glutamate side chain (E5) and the positively charged N-terminus of the next pilin subunit plays a role in attracting subunits to the assembly site. Extensive hydrophobic interactions between the α1-N regions in the filament core are believed to be responsible for pilin polymerization. The globular domains are more loosely packed on the pilus surface resulting in a corrugated pilus surface. The D region, which is delineated by conserved cysteines, is characterized by extensive antigenic variation. The GC pilin has two post-translational modifications at positions Ser63 (carbohydrate) and Ser68 (phosphate). (*C*) Protein structure of the *S. pyogenes* major pilin Spy0128, which shows a two-domain immunoglobulin-like fold. A conserved lysine at position 161 (K161) in the C-terminal domain is used to covalently cross-link the protein with the C-terminal threonine of the next subunit. The threonine is part of an EVPTG cell wall anchor motif that is also used to covalently link the pilus to peptidoglycan in the cell wall.
The structures were generated with the Swiss PDB Viewer (version 3.7) using coordinates deposited in the Brookhaven database: 1klf (FimC/FimH), 2hi2 (GC pilin), 3b2m (Spy0128).

found in UPEC strains, whereas those that bind mono-mannose groups with low affinity are prevalent on commensal *E. coli* strains [21, 22]. However, these findings have recently been questioned by Bouckaert et al. [23], who reported that FimH variants from UPEC, enterohaemorrhagic *E. coli* (EHEC) and faecal *E. coli* isolates expressed the same specificities and affinities for high-mannose structures. They also showed that high mannose glycans ending in Manα1–3Manβ1–4GlcNAc are the best FimH receptors and

concluded that the carbohydrate expression profiles of the targeted host tissues are stronger determinants of adhesion than FimH variants.

A recent study by Duncan et al. [24] revealed that FimH is not solely responsible for target receptor specificity and that the pilus rod might also play a role. They showed that expression of FimH on a heterologous pilus rod altered the binding specificity of the adhesin, suggesting that the specificity might be modulated through conformational constraints imposed on FimH by the pilus rod.

Infection by Type I pilus expressing *E. coli* leads to exfoliation of uroplakin-coated cells. This is believed to be a defense mechanism to eliminate infection. However, some bacteria are able to evade the host response by FimH-mediated invasion of bladder epithelial cells, which contributes to the frequent recurrence of UTIs in many patients [25, 26]. FimH binding to bladder cells triggers a signal transduction cascade that results in host actin reorganization, phosphoinositide-3-kinase activation and host protein tyrosine phosphorylation. The small Rho-binding proteins Cdc42 and RhoA are also required for the cell invasion process [26, 27]. It has recently been shown that the FimH receptors for bladder cell invasion differ from the receptors for cell adhesion. FimH specifically recognizes N-linked glycans on β1 and α3 integrins, which are expressed throughout the urothelium [28]. Once internalized, UPEC rapidly replicates and forms intracellular bacterial communities with biofilm-like properties, a process that requires expression of Type 1 pili by the intracellular bacteria [29]. Type I pilus expressing *E. coli* are also able to invade mast cells and macrophages after binding of FimH to the GPI-anchored receptor CD48. This process also involves subcellular lipid raft-like structures known as calveolae. It was suggested that this might result in the formation of membrane-bound vacuoles that encapsulate the bacteria and prevent phagocytosis [30].

In addition to their adherence properties, Type I pili have also been implicated in biofilm formation. *E. coli* forms biofilms on abiotic surfaces and Type 1 pili are required for initial surface attachment, a process that can be inhibited by mannose [31]. Orndorff et al. [32] have shown that aggregation of *E. coli* by secretory IgA (SIgA) is dependent on the pilus structure. Interestingly, this was independent of FimH. The pilus without the FimH adhesin also facilitated SIgA-mediated biofilm formation on polystyrene, although biofilm formation was stronger in the presence of FimH.

P pili. P pili are adhesive organelles that are critical virulence factors in the establishment of pyelonephritis by uropathogenic *E. coli* (UPEC), mediating recognition of and attachment to tissues of the kidney [33]. The P pilus is a composite organelle similar to the Type 1 pilus and is encoded by 11 **p**yelonephritis-**a**ssociated **p**ili (*pap*) genes. The 6.8 nm wide and several micrometers long rod is a right-handed helical cylinder composed of repeating PapA with 3.28 subunits per turn. The 2–3 nm thick tip fibrillum contains the distally located PapG adhesin and the three minor pilus proteins PapE, PapF and PapK [34, 35]. There are three different classes of PapG variants (PapGI, -II, and –III), which differ in their receptor specificity. All receptors bound by the PapG variants contain a common Gal(α1–4)Gal moiety linked to a ceramide group by a β-glucose residue, but differ in the number of N-acetylgalactosamine moeties or by the addition of sialic acid residues [36]. The receptors are found on human erythrocytes of the P blood group and on uroepithelial cells, and the different binding preferences of the PapG variants seem to contribute to host cell tropism of P-piliated UPEC [37]. The PapG-I variant preferentially binds globotriaosylceramide (GbO3), which is abundant on human uroepithelial cells. PapG-II binds to globoside (GbO4, a glycolipid iso-receptor of the human kidney) and is primarily associated with human pyelonephritis and bacteremia, whereas the PapG-III variant preferentially binds to Forssman antigen (globopentosylceramide, GbO5) and is associated with human cystitis [38]. Like FimH, PapG has a two-domain fold with an N-terminal Ig-like adhesin domain and a C-terminal conserved pilin domain possessing an incomplete Ig fold. The structure of the adhesin domain of PapG-II bound to GbO4 was determined by multi-wavelength anomalous dispersion and revealed an elongated jellyroll motif with only remote structural homology to the FimH adhesin domain [39]. Interestingly, the glycolipid receptor-binding site was found to be located at the side of the molecule, which requires a docking approach parallel to the cell membrane. This is probably achieved by the flexible nature of the tip fibrillum.

Afa/Dr adhesin family. The Afa/Dr adhesins are a highly heterogeneous group of homopolymeric adhesive organelles identified in UPEC and diffusely adhering *E. coli* (DAEC). They were originally identified as afimbrial structures, but several members can be assembled into pilus-like structures, which resemble the thin fibrillum at the tip of Type 1 and P pili. Others resemble afimbrial capsules surrounding the bacterial cell (reviewed in [40, 41]). The Afa/Dr adhesins are encoded by a cluster of at least 5 *afa* genes (A–E), with *afaE* encoding the actual adhesin. The structure of the adhesin determines whether the

adhesive organelle forms fimbriae (e. g. DraE and DaaE subunits) or an afimbrial structure (e. g. AfaE-III subunit) [4]. In the fimbrial structure, many copies of a receptor-binding major subunit (a single-domain adhesin) assemble into thin and flexible fibers.

Most Afa/Dr adhesins recognize the decay accelerating factor (DAF, CD55) which is expressed on erythrocytes and other tissues, including uroepithelium, and carcinoembryonic antigen-related cell adhesion molecules (CEACAMs), such as carcinoembryonic antigen (CEA) and CEACAM-1 (CD66a). DAF (CD55) is a complement-regulatory membrane protein that protects host tissue from damage by accelerating the decay of C3 and C5 convertases on epithelial cells. At least four members of the Dr adhesin family (AfaE-I, AfaE-III, DraE and DaaE) possess two binding sites and bind to both receptors independently [42]. The DraE subunit (but not the highly homologous AfaE-III) also binds to Type IV collagen. A strong positive selection for amino acid replacements was found in DraE, and variants with decreased sensitivity of DAF binding to high salt were found on isolates associated with UTIs, suggesting functional adaptation to their pathogenic niche.

Afa/Dr adhesins are believed to facilitate ascending colonization and chronic infection of the urinary tract and some members are also associated with enteric infection. In addition, they have been shown to facilitate UPEC invasion of uroepithelial cells. Purified Dr fimbriae applied to polystyrene beads were capable of triggering receptor clustering and the accumulation of actin at the adhesion sites on HeLa cells where beads were engulfed and ultimately internalized by the cells [43]. Recently, it has been reported that the AfaD adhesin also has invasin properties by specifically binding to integrin $\alpha5\beta1$ [44]. A recent study by Diard et al. [45] has shown that Dr fimbriae can be released into the extracellular medium in response to environmental signals, such as temperature and reduced oxygen, but the purpose of this mechanism remains elusive.

Other adhesive structures. The full number of known adhesive structures is too large to describe in this review. Other important structures include the S pili, Hif pili of *Haemophilus influenza*, a number of non-pilus structures like the *Yersinia pestis* F1 antigen [7] and the colonization factor antigen I (CFA/I) of enterotoxigenic *E. coli* (ETEC).

S pili are associated with *E. coli* strains that cause sepsis, meningitis and UTI. The SfaS adhesin interacts with sialic acid residues on endothelial cells and with kidney epithelial cell receptors. The major SfaA pilin also has adhesive properties and binds to endothelial cell glycolipids and plasminogen (reviewed in [46]). *Proteus mirabilis*, a common causative agent of cystitis and polynephritis, produces pili known as the *Proteus mirabilis* fimbriae (PMF). The *pmf* operon encodes 5 predicted proteins, the major pilin PmfA, the usher PmfC, the chaperone PmfD, the minor pilin PmfE, and the adhesin PmfF (reviewed in [47]. *E. coli* also produces a number of thinner fibers (2–5 nm wide), e. g. K99 pili, K88 pili, F17 pili, and F6 pili, although most of these structures are associated with animal ETEC.

Assembly by the chaperone-usher pathway. The structural mechanisms of the chaperone-usher pathway were first described for Type 1 and P pili (reviewed in [10]) (Fig. 2A). The protein structures of the pili subunits show incomplete immunoglobulin (Ig)-like structures in which the seventh (G) β-strand is missing, causing the formation of a hydrophobic groove. The subunits, which are produced in the cytoplasm and secreted into the periplasm via the Type II secretion system, are unable to spontaneously form an Ig-like structure or polymerize into a pilus fiber. Once in the periplasm, they form a stable complex with a chaperone (FimC in Type 1 pili and PapD in P pili), which protects the subunits from degradation, aggregation and premature polymerization.

The molecular basis for the chaperone – subunit interaction was revealed after X-ray analysis of the FimC-FimH complex (Fig. 1A) [17, 48]. The chaperone is composed of two Ig-like domains arranged in a boomerang-like shape. The N-terminal domain contains a G1 β-strand that completes the Ig-like structure of the pilin subunit by a mechanism known as 'donor strand complementation'. This is based on the interaction of alternating hydrophobic residues on the G1 donor strand with hydrophobic residues of the F β-strand of the subunit [49]. Structurally conserved chaperone structures and evidence for donor strand complementation have also been found in P pili [49], S pili [50], the *Haemophilus influenza* hemagglutination pilus [51], Dr adhesins [4] and non-pilus systems, such as *Yersinia pestis* F1 antigen [52] and CFA/I [53]. However, there are differences between chaperones in the number of residues that connect the G1 donor strand and the F1 strand. This has led to the identification of two groups; the FGS chaperones contain a 'short F1-G1 loop' and are associated with the assembly of heteropolymeric pili (e. g. Type 1 and P pili), whereas the FGL chaperones contain a 'long F1-G1 loop' and assist in the formation of homopolymeric structures (e. g. Dr adhesins, F1 antigen, Salmonella atypical fimbriae) (reviewed in [54].

The pilus subunits polymerize after the chaperone-subunit complex contacts an outer membrane chan-

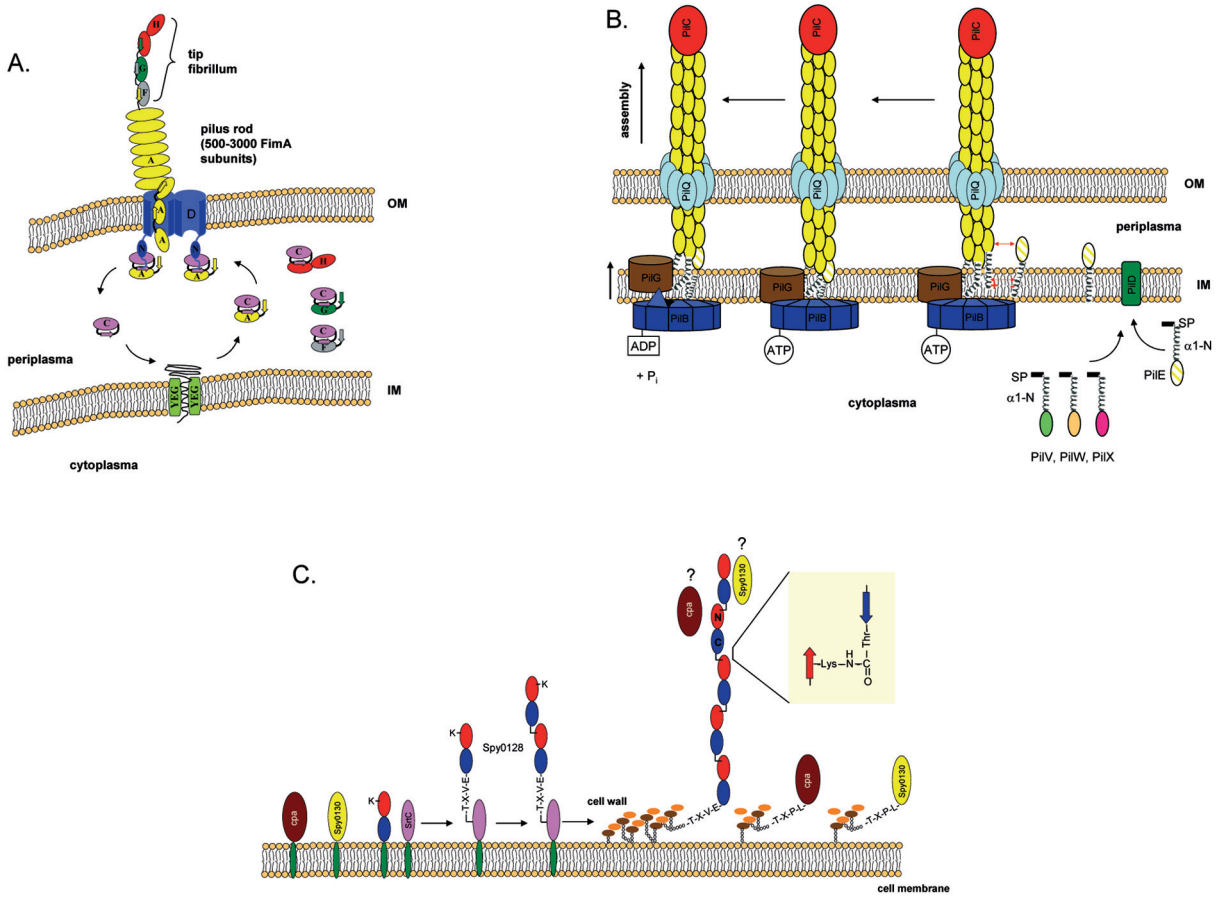

Figure 2. Assembly of various pili in Gram-negative and Gram-positive bacteria

(*A*) The *E. coli* Type I pilus as an example for a chaperone-usher pathway assembled pilus. Pilin subunits are secreted through the inner membrane (IM) into the periplasm by the Sec YEG translocon. The chaperone FimC accelerates subunit folding and stabilizes the proteins by donating a strand to complement the incomplete Ig-like fold of the subunit proteins, a mechanism known as 'donor strand complementation'. The chaperone-subunit complexes are then delivered to the FimD transmembrane assembly platform (usher), which recognizes the binary complexes by an extended N-terminal domain. The FimC chaperone is released back to the periplasm after the donor strand is replaced by an N-terminal extension of another pilin subunit, a mechanism known as 'donor strand exchange'. Although FimD forms a twin-pore complex, only one pore is used for secretion, whereas both pores are used for chaperone-pilin recruitment. The completed pilus is composed of a pilus rod of polymerized FimA subunits (yellow) and a tip fibrillum with FimF (grey), FimG (green) and the tip adhesin FimH (red). Adapted from [9]

(*B*) Assembly of the *Neisseria* GC/MC pilus as an example of a Type IV pilus. The major pilin subunits (PilA, yellow) are secreted through the inner membrane (IM) into the periplasm and the signal peptide (SP) is cleaved by the prepilin peptidase PilD (green). The negative charge of the glutamic acid side chain (E5) in the α1-N region attracts the positively charged N-terminus of the terminal PilA subunit in the growing fiber. Additional interactions between the globular PilA domains enable the subunit to fill the existing gap at the pilus base. The assembly ATPase (PilB, blue), which is associated with the cytoplasmic part of the inner membrane protein PilG (brown), undergoes conformational change during ATP hydrolysis and pushes the pilus filament out of the membrane, providing a gap for the next PilA subunit. Several minor pilins (e.g. PilV, PilX and PilX) also contain the α1-N region and can also be added to the pilus structure. In addition to the assembly ATPase PilB, the GC/MC pili apparatus also contains a structurally related retraction ATPase (PilT) that can remove pilin subunits at the pilus base resulting in 'twitching motility' (not shown). Adapted from [62].

(*C*) Homopolymerization of Spy0128 in *S. pyogenes* as an example of Gram-positive pilus assembly. Spy0128 (blue and red) is produced as a precursor molecule with a C-terminal membrane anchor (green) and the cell wall-anchor motif EVPTG, a variation of the typical LPXTG motif. A specialized sortase (SrtC, pink) recognizes the EVPTG motif and cleaves between the threonine and the glycine to generate a covalently linked sortase-pilin intermediate. The carboxyl group of the C-terminal threonine of Spy0128 is then linked to the ε-amino group of the side-chain of a conserved lysine (K161) in the N-terminal domain of the next Spy0128 subunit (yellow box). Finally, the sortase-Spy0128 intermediate is targeted by the pentapeptide of a peptidoglycan precursor to link the completed pilus to the bacterial cell wall (probably by the house keeping sortase A, not shown). The minor pilins Spy0130 (yellow) and collagen binding protein (Cpa) (brown) also contain the C-terminal cell wall-anchor domain and are covalently linked to the pilus along the shaft by an unknown mechanism. The minor pilins Cpa and Spy0130 can also be directly linked to the PG by sortase A. Adapted from [151, 152]. (N-acetyl glucosamine, brown; N-acetyl muramic acid, orange).

nel-forming protein, called the 'usher' (FimD in Type 1 and PapC in P pili) (Fig. 2A). PapC forms a twin-pore secretion complex with a central 2 nm wide β-pore in each subunit, which allows the translocation of chaperone-subunit complexes, but not the assembled pilus rod [55]. The crystal structure of a ternary complex between the soluble N-terminal FimD domain, FimC and the pilin domain of FimH revealed that the N-terminal tail of FimD recognizes FimC and is also able to discriminate between bound pilus subunits [56]. The crystal structure of the translocation domain of the PapC usher, together with single particle cryo-EM imaging of the FimD usher bound to a translocating pilus assembly intermediate, provided further insight into the twin-pore translocation process. Interestingly, only one pore is used for secretion, whereas both usher protomers are used for chaperone-subunit complex recruitment. The translocating pore is occluded by a folded plug domain and it has been suggested that it is gated by a conformationally-constrained β-hairpin [57].

Pilus assembly is achieved by a mechanism known as 'donor strand exchange'. Each pilus subunit, except the tip adhesin, contains an N-terminal extension of about 15 residues, which can replace the donor strand of the chaperone to complement the fold of the previous pilus subunit [11]. The driving force for the strand exchange is believed to lie in the preserved folding energy of the chaperone-subunit complex, where the chaperone G1 donor strand is orientated parallel to the F β-strand. During exchange, the N-terminal donor strand of one subunit is placed in antiparallel orientation to the F β-strand of the next pilus subunit resulting in a canonical Ig-fold [6]. The pilus tip adhesin initiates assembly and is the first subunit incorporated into the pilus. This is based on the higher affinity of the usher for the chaperone-adhesin complex and, as shown in the Type 1 pilus, depends on the recognition of the FimH lectin domain by FimD [58]. Furthermore, FimH triggers the conversion of FimD into a high-efficiency assembly catalyst by increasing the donor strand exchange rate between FimC-FimA complexes. The catalytic power of FimD thus lies both in acceleration of the incorporation of the first FimA subunit into the preformed tip fibrillum and in the following polymerization reaction during which FimA subunits are assembled into the pilus fiber, a process that is independent of cellular energy [59].

Type IV pili. The term Type IV pilus was originally coined in 1975 by Ottow [60]. These pili represent the most widespread class known (Table). Although mainly found in a large variety of Gram-negative bacteria, including enteropathogenic *E. coli* (EPEC), EHEC, *Salmonella enterica*, *Pseudomonas aeruginosa*, *Legionella pneumophila*, *Neisseria gonorrhoeae*, *Neisseria meningitidis*, and *Vibrio cholerae* (reviewed in [61–63]. Type IV pili have also been discovered in two Gram-positive genera (*Clostridia* and *Ruminococcus* [64, 65] and in Cyanobacteria [66].

Type IV pili are thin (6–8 nm wide), flexible fibers, several micrometers long, which often aggregate laterally to form characteristic bundles. In addition to host cell adhesion and biofilm formation, which are also characteristics of many other pili, Type IV pili possess a number of unique features, including DNA uptake during transformation, phage transduction and a flagella-independent form of movement known as twitching motility. These attributes are based on the capacity of Type IV pili to retract, generating a significant mechanical force (reviewed in [67]. In addition, Type IV pili from *Geobacter sulfurreducens* have been found to function as microbial nanowires for extracellular electron transfer [68].

Type IV pili are usually homopolymers of a single 15–20 kDa pilin subunit and, in some pili possess an adhesive subunit at the pilus tip. All Type IV pilins share some common features: a) a homologous and very hydrophobic N-terminal domain of approximately 25 residues; b) an unusual N-methyl-phenylalanine at the N-terminus; c) a pair of cysteines in the C-terminal region; and d) a conserved protein structure with an α/β-roll fold formed by the hydrophobic packing of the C-terminal half of a long α-helix against an antiparallel β-sheet. The conserved N-terminal half of the same α-helix protrudes from this globular head domain giving the pilin a 'ladle-like' shape (Fig. 1B (reviewed in [62, 63].

A characteristic of Type IV pili subunits is that they are synthesized as precursors (prepilins) with a hydrophilic leader peptide ending with a glycine, which is cleaved by a unique leader peptidase. Type IV pili can thus be classified into two groups, based on the length of the mature protein and the length of the leader peptide in the pilin precurser (prepilin). Type IVa pilins possess short leader peptides of less than 10 residues and a length of 150–160 residues, whereas Type IVb pilins have long leader peptides of 15 to 30 residues and are either long (180–200 residues) or very short (40–50 residues).

In contrast to other pili, Type IV pili biogenesis involves a large number of proteins (12 or more) that are conserved among divergent bacterial species. Several of these are core proteins that are required in all Type IV pili systems and include: a) the major pilin subunit; b) a specific inner-membrane prepilin peptidase that cleaves the N-terminal signal peptide; c) a specific ATPase that powers pilus assembly; d) an integral inner-membrane protein that recruits the

Table 1.

1. Chaperone usher pathway assembled pili

Pili	organism	major pilin	chaperone/ usher		adhesin	host cells	receptors	disease
Type 1	*E. coli*	FimA	FimC	FimD	FimH	bladder and kidney epithelial cells, buccal cells, erythrocytes, mast cells, neutrophils, macrophages	uroplakin UP1a, β1α3 integrins, laminin, CD48, collagen (type I and IV)	Cystitis, sepsis, meningitis
P	UPEC	PapA	PapD	PapC	PapG	kidney epithelial cells, erythrocytes	GbO3, GbO4, GbO5	polynephritis
S	*E. coli*	SfaA	SfaE	SfaF	SfaS	bladder and kidney epithelial cells, erythrocytes, endothelial cells	sialic acid residues plasminogen	UTI, meningitis, sepsis
Hif	*H. influenzae*	HifA	HifB	HifC	HifE	nasopharyngeal cells	unkonwn	oitis media
PMF	*P. mirabilis*	FmfA	FmfC	PmfD	PmfF	bladder and kidney epithelial cells	unknown	UTI
Dr	UPEC	DraA	DraB	DraC	DraE	bladder and kidney epithelial cells	CD55/DAF, CEACAMs	polynephritis
	DAEC					neutrophils, erythrocytes	collagen (type IV)	cystitis
Afa	UPEC	AfaA	AfaB	AfaC	AfaE	uroepithelium, erythrocytes	CD55/DAFm CEACAMs	cystitis, diarrhoea
							α5β1 integrin	
F1	*Y. pestis*	Caf1	Caf1M	Caf1A		respiratory tract epithelial cells	human IL-1β	plague

2. TypeIV pili

Pili	organisms	major pilin	putative adhesin	host cells	putative receptors	disease
a) Type IVa pili						
GCP	*N. gonorrhoaeae*	PilE	PilC	epithelial and endothelial cells	MCP (CD46), C4BP	gonorrhoea
MCP	*N. meningitis*	PilE	PilC	epithelial and endothelial cells	MCP (CD46), C4BP	sepsis, meningitis
Pa pilus	*P. aeruginosa*	PilA		epithelial cells	asialo-GM1 and GM2	pneumonia, sepsis
FT pilus	*F. tularensis*	PilE		unknown	unknown	tularemia
b) Type IVb pili						
BFP	EPEC	BfpA		epithelial cells	conflicting results	diarrhoea
TCP	*V. cholerae*	TcpA		CTXϕ phage		cholera
CFA/ III	ETEC	CofA		enterocytes	unknown	diarrhoea
Longus	ETEC	LngA		unknown	unknown	diarrhoea

3. Pili in Gram-positive bacteria

Pili	organism	major pilin	minor pilins	host cells	receptors	disease
Spa	*C. diphtheria*	SpaA	SpaB, SpaC	pharyngeal epithelial cells	unknown	diphtheria
PI-1 pilus	*S. agalactiae*	GBS80	GBS52, GBS104	pulmonary cells	unknown	neonatal sepsis and
PI-2 pilus	*S. agalactiae*	GBS1477	GBS1474, GBS1478	lung and cervical epithelial cells	unknown	meningitis
GAS M1 pilus	*S. pyogenes*	Spy0128	Cpa, Spy0130	pharyngeal epithelial cells, tonsil epithelium cells, skin keratinocytes	unknown	pharyngitis, impetigo, sepsis, toxic shock

Table 1 *(Continued)*

3. Pili in Gram-positive bacteria						
Pili	organism	major pilin	minor pilins	host cells	receptors	disease
Rrg pilus	*S. pneumoniae*	RrgB	RrgA, RrgC	lung epithelial cells	unknown	sinusitis, pneumonia
Type I	*A. naeslundii*	FimP	FimQ	tooth enamel	proline-rich salivary proteins	dental caries, peridontitis
Type II	*A. naeslundii*	FimA	FimB	various host cells	glycoproteins/ glycolipids	dental caries, peridontitis

ATPase from the cytoplasma; and e) an integral outer-membrane secretin that is necessary for the emergence of Type IV pili on the bacterial surface [62, 69]. Interestingly, these proteins have homologues in Type II secretion and archaeal flagellar systems, suggesting that they have a common origin and are variations of a macromolecular transport system [70]. In addition, a 'retraction ATPase' that is responsible for depolymerization of the pilus fiber is found in all Type IVa pili.

Type IVa pili. Type IVa pili are found on a wide range of bacteria (see Table). Due to space limitations, this review will focus on the human pathogens *Neisseria gonorrhoeae*, *Neisseria meningitidis*, and *Pseudomonas aeruginosa*.

The Neisseria pilus. Type IVa pili are found in two species of the Gram-negative genera *Neisseria*. The gonococcus (GC) pilus is found in *N. gonorrhoeae*, which causes the sexually transmitted disease gonorrhoea, whereas the meningococcal (MC) pilus is found in *N. meningitis*, the leading cause of fatal sepsis and meningitis. Both pathogens colonize human mucosal surfaces and this is initiated by binding of GC/MC pili to non-ciliated host cells [71]. The pilus fiber consists of polymerized subunits of the 17–21 kDa major pilin PilE with the PilC protein (110 kDa) located at the pilus tip and also within the bacterial membrane [72]. Purified or recombinant PilC interacts with different human epithelial cell lines, primary epithelial and endothelial cells [73]. There are two highly homologous variants of PilC (PilC1 and PilC2) and either variant is sufficient for pilus assembly in *N. gonorrhoeae* and *N. meningitidis*. However, while either variant is able to promote adhesion of *N. gonorrhoeae* to host cells, mutation of PilC1 in *N. meningitidis* results in a non-adhesive, piliated, transformation-competent strain [74].

The human membrane cofactor protein (MCP, CD46) has been reported as a cellular receptor for both GC pili and MC pili [75], however, this has recently been questioned. Transgenic mice expressing human CD46 are susceptible to meningococcal infection, in particular after intranasal challenge with piliated bacteria, suggesting that human CD46 facilitates pilus-dependent interactions at the epithelial mucosa [76]. CD46, which also serves as a receptor for several other pathogens, is a glycoprotein found on all nucleated cells and plays an important role as an inactivator of complement factors C3b and C4b deposited on self-tissue [77]. In an experiment with a human cervical cell line, adherence of piliated gonococci to the cells resulted in up to 80% reduction of CD46 on the cell surface, due to specific shedding of the protein. This shows that adherence enables the pathogen to manipulate the host cell environment, although the exact role of the CD46 shedding remains elusive [78]. In addition, phosphorylation of CD46 by the Src family tyrosine kinase c-yes upon interaction with piliated gonococci has been reported, suggesting that binding of GC pili to CD46 may not simply be a static process, but might promote signaling in the host cell that could be important for bacterial virulence [79]. On the other hand, Tobiason et al. [80] have shown an inverse correlation between gonococcal adherence and surface expression levels of CD46, suggesting that CD46 does not act as a classic receptor for GC pili. The role of CD46 as pilus receptor has also been questioned by Kirchner et al., who reported CD46-independent binding of GC pili to human epithelial cells [81].

The N-terminal part of PilC also binds to the α-chain of the human complement regulator C4B-binding protein (C4BP). CD46 competed with C4BP for binding to pili only at high concentrations, suggesting that different parts of PilC are involved in these two interactions [82]. Another GC/MC pilus component, the PilE subunit-like protein PilV, was demonstrated to be essential for pilus-mediated cell adherence, but dispensable for pilus biogenesis and other pilus-related phenotypes. However, its function in adhesion appears to be indirect by promoting the functional display of the PilC adhesin in the context of the pilus fiber [83]. Involvement of PilV in post-translational modification of the major pilin PilE has also been proposed [84]. The GC/MC pilus also binds to erythrocytes causing hemagglutination, and this has

been attributed to the major pilin PilE but not the PilC adhesin [73].

After the initial pilus-mediated attachment to host tissue, the bacteria form microcolonies and induce the formation of cortical plaques, which are enriched in both components of the cortical cytoskeleton and a subset of integral membrane proteins. Cortical plaque formation depends not only on the presence of the pilus fiber and PilC, but also on expression of the pilT protein [85]. PilT is an inner membrane-associated ATPase that is involved in pilus retraction and is also required for force-dependent pilus elongation enabling the bacteria to modulate interactions with surfaces by controlling the tension on their pili [86]. Pilus retraction is required for a specialized form of movement called twitching motility that mediates bacterial movement towards the host cell surface for intimate attachment [87, 88]. The PilT-mediated twitching motility is also required for the high level competence for DNA transformation in *Neisseria* [89]. PilX, an 18 kDa pilin-like protein also plays critical roles in Type IV pili biology. It was demonstrated that, although not required for pilus assembly, PilX is co-localized with the fiber and is necessary for aggregate formation and adhesion to host cells without affecting PilC [90].

The major pilin PilE undergoes extensive antigenic variation. This is mediated by non-reciprocal recombination, in which silent partial pilin genes (*pilS*) are transferred to an active pilin expression locus by a RecA-dependent mechanism [91]. In addition, the GC/MC pilus undergoes phase variation, which results in a reversible change between piliated and non-piliated bacteria. It was shown that pilus phase variation correlates with an on/off switch in PilC expression, suggesting that PilC is also involved in pilus assembly [92]. Non-piliated GC pilus variants are characterized by formation of primarily S-pilin, a polymerization-deficient pilin monomer which was implicated as being crucial for establishment of gonococcal infections [93].

Four different types of post-translational modification of PilE have been described: a) glycosylation at Ser63; b) O-linked phosphoethanolamine (PE); c) O-linked phosphorylcholine (PC); and d) phosphorylation of Ser68 in the GC pilin. The glycosylation pattern appears to differ between GC and MC pili. It was reported that PilE in GC carries the disaccharide Gal ($\alpha 1-3$) GlcNAcN, whereas the MC pilin is modified by the unusual trisaccharide Gal($\beta 1-4$)Gal($\alpha 1-3$) 2,4-diacetamido-2,4,6-trideoxyhexose [94]. However, PilE modification with the disaccharide has also been reported in certain MC strains [95]. More recently, an alternative pilin modification was found in MC and identified as glyceramido acetamido trideoxyhexose [96]. The physiological function of pilin modification is not entirely clear, but a role in host immune evasion seems likely. It was reported that naturally occurring antibodies against the terminal Gal moiety may interfere with complement-mediated lysis [97]. Furthermore, phosphoethanolamine (PE) modification of lipopolysaccharide (LPS) has been documented in a number of important pathogens and has been implicated in resistance to cationic microbial peptides [98] and recognition by complement component C4b [99]. Phosphorylcholine (PC) was proposed to impact host-microbe interaction by serving as a ligand for both C-reactive protein (CRP) and the receptor for platelet-activating factor [100, 101]. In addition, it has been suggested that these modifications may also influence pilus structure and function, and may also provide a means for the organism to fine tune pilin membrane trafficking events [84].

Crystal structure analysis of PilE at 2.6 Å resolution (Fig. 1B) revealed a 'ladle-like' structure with an $\alpha-\beta$ roll fold, an 85 Å α-helical spine and an O-linked GlcNAc-α1,3-Gal group at Ser63 [102]. The C-terminal part of the α-helix (α1-C) is embedded in the globular head, whereas the N-terminal (α1-N) part forms the 'ladle handle'. A disulfide bond between Cys121 and Cys151 generates the disulfide loop region (D region), a highly immunogenic region that builds the basis for the extensive antigenic variation in the GC/MC pilus. Key residues were identified that stabilize interactions between D-region β-strands and connectors to allow for sequence hypervariability. Recently, the GC pilus structure was solved to 12.5 Å resolution by cryo-electron microscopy and iterative helical space reconstruction [103]. This showed that the fiber is composed of spiraling three-helix bundles, which form the filament core, and is held together by extensive hydrophobic interactions among the N-terminal α-helices within the fiber core. The globular domains are more loosely packed on the fiber surface, with protruding hypervariable loops and posttranslational modifications that shield conserved functional residues in pronounced grooves resulting in a highly corrugated fiber surface. These grooves are often rich in positively charged residues, which might explain the role of the pilus in DNA uptake.

Pseudomonas aeruginosa pilus. P. aeruginosa is a Gram-negative opportunistic pathogen that is a major cause of nosocomial infections and a leading cause of hospital-acquired pneumonia. It also causes bacteremia in burn victims and severe persistent respiratory infection in susceptible individuals, in particular cystis fibrosis patients [104].

P. aeruginosa produces Type IV pili very similar to the GC/MC pili, which extend as bundles from one or both

of the cell poles. These are involved in colonization during infection, twitching motility, biofilm formation, bacteriophage infection, and natural transformation. Fiber diffraction studies on the *P. aeruginosa* strain K and strain O pili (PAK pili and PAO pili) revealed hollow cylinders with five pilin subunits per helical turn, a 52 Å outer diameter, a 12 Å inner diameter and a ~31 Å diameter ring of hydrophobic residues [105]. The pili are the dominant adhesins in *P. aeruginosa* and specifically bind to the β-GalNAc(1–4)βGal moiety of glycolipids asialo-GM1 and asialo-GM2 on epithelial cells [106]. Binding to host cells through the *P. aeruginosa* pilus is a tip-associated event that is mediated by the C-terminal region of the 15 kDa structural subunit PilA [107]. The receptor-binding domain (disulfide-loop region) shows significant sequence variation among different *P. aeruginosa* strains, but this has no effect on receptor recognition [108]. The importance of pili in the binding of *P. aeruginosa* to epithelial cell surfaces has been shown by a 90% decrease in the ability of non-piliated strains of *P. aeruginosa* to bind human A549 type II pneumocytes [109].

Within *P. aeruginosa* species, five distinct variants of the structural subunit PilA, varying in amino acid sequence, length, and presence of posttranslational modifications have been identified (group I to V pilins). An O-linked trisaccharide covalently attached through the β-carbon of the C-terminal serine residue was found on the group I *P. aeruginosa* strain 1244 pilin. The glycan was identified as α-5NβOHC$_4$7NFmPse-(2–4)β-Xyl-(1–3)-β-FucNAc-(1–3)-β-Ser and has the same sugar composition and sequence as the O-antigen repeating unit of *P. aeruginosa* 170046, a strain belonging to the LPS O7 serotype. This suggests a common biosynthetic origin between pilin glycosylation and O-antigen production [110]. The glycan modification on the pilin of the group IV strain Pa5196 was determined to be an unusual homooligomer of α-1,5-linked D-arabinofuranose (D-Ara*f*) O-linked to threonine residues, a sugar that occurs mainly in the cell wall arabinogalactan and lipoarabinomannan (LAM) polymers of mycobacteria [111].

Type IVb pili. Type IVb pili are mainly found on bacteria associated with human intestinal infections, e. g. EPEC, ETEC, *Salmonella typhi* and *Vibrio cholerae*. In this review, we will focus on the bundle-forming pilus (BFP) of EPEC and the toxin co-regulated pilus (TCP) of *V. cholerae*.

Bundle-forming pilus (BFP) of EPEC. Enteropathogenic *Escherichia coli* (EPEC) is a major cause of infant diarrhoea in developing nations [112]. In addition to Type 1 pili (see above), EPEC carry Type IV pili known as the bundle-forming pili (BFP), in recognition of their ability to associate and form intertwined rope-like aggregates. These structures are over 15 μm long and have a polar localization on the cell. BFP-mediated inter-bacterial interactions are responsible for the formation of three-dimensional microcolonies on the surface of epithelia [113]. This has been referred to as localized adherence (LA) and is an important step in colonization. During adhesion, BFP organize into higher-order cables of increasing diameter and strength [114]. Bundlin is the major pilin of BFP and is encoded by the plasmid-borne *bfpA* gene, which is the first cistron of the 14-gene *bfp* operon. Both cable formation and dispersal of aggregates requires the cytoplasmic nucleotide binding protein BfpF [114].

There are conflicting results on the role of BFP as a host cell adhesion, and several different BFP receptors have been proposed. Jagannatha et al. [115] reported EPEC strains that show LA binding to asialo-GM1, asialo-GM2, globoside and lacto-N-neotetraose, based on recognition of GalNac-β-1–4 Gal groups. Other groups reported inhibition of LA binding by N-acetyl-galactosamine [116], N-acetyllactosamine and LewisX [117]. More recently, recombinant bundlin was shown to inhibit LA to HEp-2 cells. Bundlin also bound to HEp-2 cells and, with millimolar association constants, to synthetic N-acetyllactosamine as shown by nanoelectrospray ionization mass spectrometry [118]. Another group showed that purified BFP was able to bind phosphatidylethanolamine (PE), and that this interaction was inhibited by antibodies against bundlin [119]. In contrast, Hicks et al. [120] found that there were no differences in initial adherence between a wild-type EPEC strain and a *bfp*- mutant. They proposed that other factors, including intimate attachment via intimin leads to the initial 'attaching and effacing' phenotype, whereas BFP are involved in the recruitment of additional bacteria to form the three-dimensional micro-colonies.

The structure of bundlin (BfpA) was recently analysed by NMR and revealed a general Type IVb pilin architecture, with the C-terminal segment forming the central strand of the β-sheet. However, the structure also showed significant differences in the composition and relative orientation of secondary structure elements [121].

Toxin co-regulated pilus (TCP) of Vibrio cholerae. V. cholerae is the aetiologic agent of cholera, a devastating dehydrating diarrhoea caused by toxin production of bacteria colonizing the mucosa of the human small intestine. A critical colonization factor of *V. cholerae* is the toxin-co-regulated pilus (TCP), as TCP-deficient

V. cholerae mutants are incapable of colonizing the intestines [122].

The TCP structure is assembled as a homopolymer of repeating subunits of TcpA pilin. Like the BFP in EPEC, TFP are polar structures of more than 15 μm in length that self-aggregate, bringing the bacteria together in microcolonies. The primary structure of TcpA is highly conserved among *V. cholerae* serogroups and biotypes shown to be pathogenic to humans, which is in sharp contrast to the antigenic variation seen in other Type IV pili, such as the *Neisseria* pili.

The crystal structure of a soluble, monomeric form of TcpA, without the hydrophobic N-terminal 28 residues was solved to 1.3 Å resolution [123]. In the known PAK and GC pilin structures, this segment forms the protruding N-terminal half ('ladle handle') of an extended α-helix. Morphologically, TCP appears as long bundles of laterally associated pilus fibers most similar to those of the bundle-forming pilus (BFP) found in EPEC. However, the monomer subunits (TcpA and BfpA) display distinct differences and cannot complement each other for pilus assembly [121]. Analysis of the TCP structure by hydrogen/deuterium exchange mass spectrometry revealed a tight packing of the N-terminal α-helices of TcpA (the 'ladle handle'), but loose packing of the C-terminal globular domains. This results in substantial gaps, with exposed glycine-rich amphipathic segments on the pilus surface [124]. The authors proposed that the structure explains the extreme flexibility of the pilus fiber and suggests a molecular basis for pilus-pilus interaction.

The *tcp* genes that encode the pilin and TCP biogenesis functions are organized as an operon located on the 39 kbp *Vibrio* pathogenicity island (VPI) [125]. The acquisition of VPI by *V. cholerae* endows the organism with the ability to express TCP, which acts as a receptor for the cholera toxin gene carrying lysogenic bacteriophage CTXϕ [126]. Although it has been suggested that TCP is involved in epithelial cell adhesion, no specific receptors have been identified and the mechanism by which TCP mediates colonization and whether or not TCP is the only factor required for colonization still remain elusive.

Structure and Assembly of Type IV pili. In contrast to the chaperone usher pathway, used for the assembly of Type I and P pili, the mechanism of Type IV pilus assembly is only poorly understood. Here, we use the nomenclature for the GC/MC pilus component proteins (see also Fig. 2B). Individual pilin subunits are synthesized as precurser molecules (prepilins) in the cytosol and translocated across the inner membrane by the Sec machinery followed by folding of the globular domain and the introduction of stabilizing intramolecular disulfide bonds by an oxidoreductase enzyme (DsbA) [127]. The N-terminal leader sequence is then cleaved by a dedicated prepilin peptidase (PilD), which also adds a methyl group to the N-terminal amine [128].

Pilus assembly occurs from a molecular platform and requires ATP hydrolysis. The platform consists of an inner membrane protein (PilG) and an assembly ATPase (PilB). Analysis of recombinant *N. meningitidis* PilG by electron microscopy revealed that PilG exists as a tetramer with an asymmetric bilobed structure that allows contact with both cytosolic and periplasmic proteins [129]. PilB is a hexameric ATPase that is recruited to the cytosolic lobe of the PilG inner transmembrane protein by an integral membrane protein [130]. Retractile pili also require a retraction ATPase (PilT) for rapid depolymerization of the filament, which confers twitching motility and allows the bacteria to move along semi-solid surfaces, to transduce phages and to take up DNA (reviewed in [131]). Both ATPases belong to the Type II/IV secretion NTPase superfamily.

The recent structural analysis of a related ATPase, PilT from *Aquifex aeolicus*, revealed that motility is based on large domain movements and suggests that different binding states can exist in a single PilT hexamer and that conformational changes in one subunit might affect neighbouring subunits [132]. Based on these and other findings, Craig and Li [62] have recently proposed a model for Type IV pilus assembly in which: a) pilin subunits are suspended in the inner membrane via their hydrophobic N-terminal α-helices (α1-N region); b) complementary interactions between a conserved negatively charged side chain (Glu5 in the α1-N region) and the positively charged N-terminal residue, and additional interactions between discrete regions of the globular domain, contribute to docking the pilin subunits into the growing pilus fiber; c) the filament is extruded into the periplasm due to the mechanical force generated from a single ATP hydrolysis event; and d) pilin subunits are added one at a time, but at three sites simultaneously corresponding to each filament strand in the three-helix bundle.

Finally, the assembled pilus structure has to pass through the outer membrane, which is achieved by the outer membrane secretin (PilQ), a member of the secretin superfamily that is also utilized in Type II and Type III secretion systems and in filamentous phage release [133]. PilQ has a cage-like structure with four-fold symmetry, consistent with a dodecamer comprising a tetramer of PilQ trimers [134]. The PilQ complex is a gated channel that requires substantial conformational change to allow an intact pilus filament to pass through.

Curli pili

Curli were first described in 1989 as proteinaceous coiled fibers found on enteric bacteria such as *E. coli* and *Salmonella spp.* [135]. They consist of repeating subunits of the major pilin CsgA (or curlin) that lacks structural homology to any other known pilin protein. Interestingly, curli share some biochemical and structural properties with the eukaryotic amyloid fibers found in some neurodegenerative diseases, such as Alzheimer's and Parkinson's disease, and in prion diseases [136]. They are non-branching, β-sheet rich fibers that are resistant to protease digestion and 1% sodium dodecylsulfate (SDS) [137]. However, unlike eukaryotic amyloid fibers, curli are produced by a highly regulated process and not all *E. coli* strains that encode the structural subunit can assemble it into a fiber [135].

Curli have been implicated in a number of biological processes, including biofilm formation, cell aggregation, host cell adhesion and invasion, and as potent inducers of the host's inflammatory response. Both *E. coli* and *Salmonella spp.* curli are important for biofilm development. Austin et al. [138] have demonstrated that curli allow *Salmonella enteriditis* to adhere to Teflon and stainless steel and have suggested that the persistence of the bacteria on these surfaces could have implications for the food industry. Curli bind to several host proteins, but their role in pathogenesis is not entirely clear, as it is believed that curli assembly preferentially occurs at temperatures below 30°C [135]. However, curli expression at 37°C has also been reported [139].

Curli bind to the extracellular matrix protein fibronectin [135], which might assist in host colonization. Simultaneous binding to plasminogen and tissue type plasminogen activator (t-PA), resulting in conversion of plasminogen to the active plasmin, has also been reported. Plasmin is an anticoagulant that degrades fibrin in blood clots, which facilitates bacterial spreading. Herwald et al. [140] reported the binding of fibrinogen and contact phase proteins such as H-kininogen, and factor XII to curli on the bacterial cell surface. As a consequence, the proinflammatory pathway is activated through the release of bradykinin, a potent inducer of fever, pain and hypotension. Absorption of these proteins by bacterial surface proteins depletes relevant coagulation factors, which results in delayed blood clotting, facilitating bacterial spreading. The authors suggested that the interplay of microbe surface proteins and host contact-phase factors may contribute to the symptoms of sepsis and septic shock. Curli have also been characterized as pathogen-associated molecular patterns (PAMPs) that are recognized by toll like receptor 2 (TLR2), resulting in activation of the innate immune response by stimulation of IL-8 expression [141].

Curli are assembled via the nucleation precipitation pathway model [142]. In this model, soluble CsgA (curlin) and CsgB are secreted into the extracellular milieu with the help of an outer membrane lipoprotein, CsgG. This process also requires two accessory proteins, CsgE and CsgF, which both interact with CsgG and facilitate CsgA secretion and assembly. Outside the cell, CsgA is nucleated into a fiber by CsgB.

CS1 pilus family

CS1 pili are the prototype of a family of serologically distinct pili associated with enterotoxigenic *E. coli* (ETEC), which also includes CFA/I, CS2, CS4, CS14, CS17 and CS19 (reviewed in [143]) and are sometimes classified as alternative chaperone usher family of pili, Class 5 or α-fimbriae. ETEC is a major cause of human diarrhoeal disease worldwide and is responsible for both traveller's diarrhoea and significant mortality among infants and young children in developing countries [144]. Only four structural and assembly proteins are required to produce fully functional pili. The 15.2 kDa CS1 major pilin, CooA, lacks any significant sequence homology to other pilins and also lacks cysteine residues to form the typical intramolecular disulfide bridges found in Type IV pilins and pilins in chaperone usher pathway assembled pili [145]. CooD is a 38 kDa minor pilin associated with the CS1 pilus tip. It has been shown that alanine conversion of a single CooD residue (Arg181) abolished hemagglutination without affecting pilus assembly, suggesting that CooD might be essential for CS1-mediated host cell attachment [146]. However, CooD is also required for pilus morphogenesis, as CS1 pili are not produced in the absence of CooD [147]. CooB is a 28 kDa protein and has an essential role in pilus assembly, although it is not associated with the pilus fiber. CooB has a chaperone-like function through which it stabilizes CooA, CooC and CooD in the periplasm [148]. Despite the lack of homology between CS1 and Pap-related pili, their assembly shares some common features. CS1 pili are assembled by the 'alternative chaperone usher pathway'. Sekellaris and Scott [143] have proposed a model for pilus assembly in which a CooD-CooB complex finds a free CooC-CooB complex in the outer membrane to initiate assembly, releasing the CooB chaperone. A CooB-CooA complex then displaces CooD from CooC to replace it with CooA. A repeated process would allow incorporation of CooA at the pilus base resulting in extension of the pilus structure.

Pili in Gram-positive bacteria

Pilus structures in Gram-positive bacteria were first described in 1968, when an electron-microscopy study showed flexible rods on the surface of *Corynebacterium renale* [149]. Since then, pili have been discovered in many other Gram-positive bacteria, including *Actinomyces*, *Ruminococcus*, *Enterococcus*, *Clostridia*, and several species of *Streptococcus* (reviewed in [150, 151]. Two types of pili in Gram-positive bacteria have been identified by electron microscopy. Short, thin rods that extend between 70 and 500 nm in length, with diameters of 1–2 nm have been found on the surface of *Streptococcus salivarius*, *S. gordonii* and *S. oralis* (Willcox, 1989). Much longer flexible pili (0.3–3 μm) with diameter of 3–10 nm have been described in *Corynebacterium spp*. and pathogenic streptococci, such as *S. pneumoniae*, *S. agalactiae* and *S. pyogenes*. These are composed of multiple copies of a single backbone pilin subunit that forms the pilus shaft, together with accessory pilins, or minor pilus proteins, which are not required for pilus integrity, but might function as adhesins.

The first insights into the assembly mechanism of Gram-positive pili were provided by Thon-That and Schneewind working on *Corynebacterium diphteriae* [152]. They showed that, in contrast to Gram-negative pili, the major pilus subunits are connected covalently and the pilus rod is then anchored to the cell wall by a sortase enzyme (a specialized transpeptidase), similar to the cell wall anchoring of proteins referred to as 'Microbial Surface Components Recognizing Adhesive Matrix Molecules' (MSCRAMMs) (reviewed in [153]. A Gram-negative-like Type IV pilus structure conferring gliding motility was also found in *Clostridium perfringens* [64] and in *Ruminococcus* [65].

Pili in *Corynebacterium diphtheriae.* *C. diphtheriae* colonizes the human nasopharynx or skin, causing diphtheria, a rapidly developing acute and feverish infection (reviewed in [154]). The pili in *C. renale* were the first pili discovered in Gram-positive bacteria [149]. *C. diphtheriae* is able to produce three different pili that are named according to their major subunit, **s**ortase-mediated **p**ilin **a**ssembly (Spa) A, D, or H [155]. Immunogold electron microscopy of the SpaA pilus and experiments with deletion mutants revealed that SpaA builds the pilus shaft, SpaC is located at the pilus tip and SpaB is associated along the pilus length [152]. *C. diphtheriae* encodes six sortase genes (*srtA-F*) and several, but not all, are required for precursor processing, pilus assembly or cell wall envelope attachment. Deletion of the pilus shaft results in the display of the minor pilins SpaB and SpaC on the cell surface, a process dependent on sortase A (SrtA). A recent study provided evidence that the minor pilus proteins SpaB and SpaC are involved in specific colonization of human pharyngeal cells. Antibodies against SpaB or SpaC blocked bacterial adherence and latex beads coated with SpaB or SpaC specifically bound to pharyngeal cells [156]. A strain that expresses only the SpaA-type pilus adheres well to pharyngeal cells, but lacks significant binding to any other cells, indicating a role for pili in tissue tropism.

Pili in pathogenic streptococci. Pili in pathogenic streptococci appear as long, flexible rods protruding up to 3 μm from the cell surface. The recent completion of whole genome sequences from *S. pneumoniae*, *S. agalactiae* and *S. pyogenes* has led to the discovery of pilus component proteins and assembly sortases and has shed some light on pilus biogenesis.

Streptococcus agalactiae. S. agalactiae (group B streptococcus, GBS) is the major cause of neonatal sepsis and meningitis in the developed world and causes invasive disease in the elderly (reviewed in [157]).

From analysis of the complete *S. agalactiae* genome, Lauer et al. [158] discovered a gene cluster (pilus island 1, PI-1) encoding two predicted sortases (SAG0647 and SAG0648) and three proteins with cell wall-anchoring motifs (GBS52, GBS80 and GBS104). Using immunogold EM they showed that the structural proteins build a pilus structure, in which GBS80 forms the pilus shaft, whereas GBS52 and GBS104 are minor pilins associated to the pilus fiber. This was also confirmed by Western blot analysis, which showed high molecular weight polymers, an indication of covalently polymerised pilin subunits. A related pilus-encoding gene cluster (PI-2), of which there are two variants, was found in the genomes of eight out of eight sequenced *S. agalactiae* strains and each of them was shown to encode all necessary genes for pilus production [159]. A potential role of the minor pilus proteins as adhesins has recently been demonstrated by Dramsi et al. [160], who showed that deletion of *gbs1474* (a paralogue of *gbs52*) in strain NEM316 resulted in significant reduction of adherence to lung and cervical epithelial cells. However, GBS1474 is located mostly at the pilus base and at random along the pilus shaft, and mutants devoid of the pilus shaft protein showed normal adherence. Similarly, deletion of *gbs52* or *gbs104* significantly reduced bacterial adherence to pulmonary cells, whereas deletion of *gbs80* resulted in pilus-deficient, but fully adhesive bacterial cells [161]. This suggests that these pili may not be necessary for host cell adhesion and that the minor pilins act as adhesins independent of the pilus structure. A recent crystal

structure of GBS52 revealed a typical adhesin fold with two immunoglobulin-like domains [161].

Streptococcus pyogenes. S. pyogenes (group A streptococcus, GAS) primarily colonizes the nasopharynx and skin and this can result in pharyngitis and subcutaneous skin diseases, such as impetigo and cellulitis, and can also lead to invasive diseases, such as necrotizing fasciitis and streptococcal toxic shock syndrome (reviewed in [162]). Using a genome mining approach in search of novel pilus gene clusters in *S. pyogenes*, Mora et al. [163] identified a gene encoding a novel sortase enzyme (SrtC) flanked by two uncharacterized proteins with predicted cell wall anchor domains. The three genes are part of a highly polymorphic gene cluster that also encodes fibronectin-binding proteins (PrtF1, PrtF2), collagen-binding protein (Cpa) and the T antigen (FCT region) [164]. Immunogold EM and immunoblot analysis revealed that the pilus shaft was formed by multiple copies of the major pilin, which is encoded by a highly variable gene located immediately upstream of *srtC*: Spy0128 (serotype M1 strain), Tee6 (serotype M6 strain), Orf80 (serotype M3, M5, M18, and M49 strains), or EftL-SL.A (serotype M12 strain). Two minor pilins were detected along the pilus shaft in all investigated *S. pyogenes* strains [163]. One was encoded by the highly variable gene immediately downstream of srtC: Spy0130 (serotype M1 strain), Orf82 (serotype M3, M5, M18, and M49 strains), or Orf2 (serotype M12 strain). The second was the collagen-binding protein Cpa, which is also encoded in the FCT region. However, no FCT region encoded fibronectin-binding proteins were found associated with the pilus.

The first crystal structure of a Gram-positive pilus shaft subunit, Spy0128, revealed an extended structure comprising two Ig-like domains (Fig. 1C) [165]. The Spy0128 structure also revealed two intramolecular isopeptide bonds (one per domain) formed between the ε-amino side chain of a lysine residue and the δ-carboxy amide group of an asparagine, in an autocatalytic process involving a nearby glutamic acid residue. These internal Lys-Asn isopeptide bonds are not only highly conserved within the *S. pyogenes* major pilus proteins, but have now also been identified in the *S. agalactiae* minor pilin GBS52 and in the collagen-binding adhesin of *Staphylococcus aureus*. Furthermore, they have been predicted to exist in other Gram-positive pilus proteins [165]. Spy0128 mutants devoid in isopeptide bonds show a significant loss in trypsin-resistance and thermal stability, indicating an important role in pilus integrity.

S. pyogenes pilus-negative mutants were shown to possess an impaired capacity to attach to Detroit-562 pharyngeal cells [166]. Recombinant forms of the serotype M1 minor pilins Cpa and Spy0130, but not the major pilin Spy0128, bound to the pharyngeal cells, suggesting that Spy0128 forms a non-adhesive pilus shaft that carries the two minor pilins acting as adhesins. Pilus-mediated attachment of *S. pyogenes* to human tonsil epithelial cells and skin keratinocytes has also been reported [167]. Furthermore, *S. pyogenes* pili were shown to contribute to bacterial cell aggregation in liquid culture, the formation of microcolonies on human cells, and the formation of biofilms [166]. Pili also contribute to GAS cell aggregation in saliva. This is mediated by the saliva component Gp340, which binds to GAS pili and reduces bacterial adhesion to human epithelial cells. It was suggested that this process might play a role in host defence [168].

Streptococcus pneumoniae. S. pneumoniae disease is triggered by colonization of the nasopharynx and includes major respiratory tract infections, such as otitis media, sinusitis and pneumonia (reviewed in [169]. Pili have been detected in some, but not all *S. pneumoniae* strains. This is consistent with the localization of pilus genes on a pathogenicity islet (*rlrA*), which may be a mobile element. *RlrA* encodes three sortases (SrtB, C and D) and three pilus component proteins: the major pilus shaft protein (RrgB) and two minor pilus proteins (RrgA and RrgC). RrgC was found at the pilus tip, whereas RrgA was located mainly at the pilus base, but also distributed along the pilus shaft [170, 171]. The introduction of the *rlrA* islet into an encapsulated *rlrA*-negative isolate produced functional pili on the cell surface and resulted in enhanced adherence to lung epithelial cells. A pilus-expressing clinical isolate was more virulent than a non-piliated deletion mutant and out-competed the mutant in murine models of colonization, pneumonia and bacteremia [171]. Furthermore, piliated *S. pneumoniae*, but not non-piliated mutants, evoked elevated levels of tumor necrosis factor-α (TNF-α) and interleukin-6 (IL-6) and it was proposed that this inflammatory response might damage the mucosal barrier and facilitate invasion.

Recently, Hilleringmann et al. [172] used cryo-electron microscopy and data from freeze drying/metal shadowing techniques to show that the pili appear to be formed by at least two protofilaments arranged in a coiled-coil compact superstructure. Triple immunoelectron microscopy showed that purified pili contained RrgB as the major compound, followed by clustered RrgA and individual RrgC molecules on the pilus surface

Pili in *Actinomyces naeslundii.* *A. naeslundii* is a human pathogen found in the oral cavity or in dental

plaque and can cause caries and periodontitis. The structurally different Type 1 and Type 2 pili are necessary for the colonization of the oral cavity. Both pili are heteromeric structures with a major pilin building the pilus shaft (FimP in Type 1 and FimA in Type 2), together with a minor pilin (FimQ and FimB, respectively) that is localized primarily at the pilus tip [173]. Type I pili adhere to the proline-rich salivary proteins of the tooth enamel, facilitating oral colonization [174]. Type 2 pili have lectin-like binding activity for glycoprotein or glycolipid receptors on various host cells. In addition they bind to cell wall glycans of some oral streptococci to form biofilms (dental plaques) [175].

Assembly of Gram-positive pili. Like certain Gram-positive adhesins, the pilus subunit protein precursors contain an N-terminal signal peptide for Sec-dependent secretion and a C-terminal cell anchor domain consisting of a membrane-spanning region preceded by the sortase recognition motif LPXTG, or a variant thereof (reviewed in [176]. After secretion of the N-terminal part and removal of the signal peptide, the protein remains anchored to the cell membrane via the hydrophobic cell anchor domain. In a following step, the sortase enzyme (a membrane-associated transpeptidase) specifically cleaves the LPXTG motif between the threonine (T) and the glycine (G) residue to form an acyl-enzyme intermediate, which is released after nucleophilic attack by the amino group of a terminal amino acid residue in the peptidoglycan precurser lipid II. As a result, the pilus protein is covalently attached to the cell wall via its C-terminus, similar to the anchoring of MSCRAMMs. This mechanism does not explain the polymerization of the pilus subunits to form the pilus fiber, however.

The first insights into the assembly of pili in Gram-positive bacteria were provided by Schneewind and colleagues, working on the pili in *C. diphtheriae* [152]. They noticed that a specific amino acid sequence, WXXXVXVYPKN (where X denotes any amino acid), which they named the 'pilin motif' is conserved in a number of major pilus proteins, including SpaA of *C. diphtheriae*. Replacement of the lysine (K) in the pilin motif of SpaA abolished the polymerization of the pilus shaft subunits. They thus proposed that the shaft subunits are connected to each other by covalent bonds joining the lysine side chain to the C-terminus of the next pilin subunit. SrtA, a SpaA pilus cluster-encoded sortase, is responsible for this polymerization process, whereas SrtF, the so-called housekeeping sortase, anchors the polymer into the cell wall. This has been demonstrated in a multiple deletion mutant that expressed SrtA as the only sortase, resulting in secretion of SpaA polymers into the culture medium. Furthermore, a mutant that only expressed SrtF displayed cell wall-anchored pilins, but no polymers [177].

Further evidence for the involvement of pilin lysine residues in pilus assembly has recently been provided for the *S. pyogenes* backbone pilin Spy0128 (Fig. 2C). Although Spy0128 lacks a sequence similar to the *C. diphtheriae* pilin motif, an invariant lysine (Lys161) was found in all Spy0128 variants. Mass spectral peptide fingerprinting with purified pili from a *S. pyogenes* M1T1 strain revealed a non-linear peptide fragment in which the ε-amino group of Lys161 was joined to the carboxyl group of the C-terminal Thr311 of another subunit [165]. The crystal structure of Spy0128 further showed that, in the crystal, molecules of Spy0128 were assembled head-to-tail in columns, with the sidechain of Lys161 close to the C-terminus of the next molecule in the column. This provided a very attractive structural model for a pilus fibre assembly. It is still not known how the minor pilins attach to the pilus shaft. Immunoblot analysis with cell wall extracts showed HMW polymers when developed with minor pilin-specific antibodies, indicating a covalent attachment. Minor pilins possess the C-terminal hydrophobic cell wall anchor domain including the LPXTG sortase recognition motif (or a variant thereof), but lack a recognisable SpaA-like pilin motif and are unable to homopolymerize in the absence of major pilin. Attachment of the C-termini of minor pilin subunits to the conserved lysine side chain seems unlikely, as this would lead to abrogation of pilus growth, unless the minor pilins also contain lysine residues that would enable pilus growth to continue. Sortase specificity appears to play a role in the incorporation of minor pilins. Pilus gene clusters often encode several different sortases. For example, the *S. agalactiae* sortases SAG0647 and SAG0648, both encoded in the pilin island 1, are responsible for the specific incorporation of GBS52 and GBS104, respectively [159]. Another conserved region in the major pilin, called the E box (due to a highly conserved glutamic acid residue) has been suggested to play a role in the attachment of minor pilins, as replacement of this glutamic acid residue in SpaA prevented incorporation of SpaB into the *C. diphtheria* pilus structure. The exact mechanism remains elusive, however [155]. Notably, minor pilins are not always linked to the fiber, but can also directly be attached to the peptidoglycan by a housekeeping sortase and function as cell wall-anchored adhesins [161].

Vaccine development

Due to their essential role in colonizing host tissue, the components of pili have long been regarded as potential targets for vaccine development. One of the first reports to describe the use of pili as vaccine candidates was published by Brinton and colleagues in 1978 [178]. They showed that purified GC pili induced antibodies that could inhibit epithelial attachment of live GC. Unfortunately, only low antibody titers were achieved and the bacteria were also able to evade immunity by antigenic variation in the pilin.

More promising results have been achieved in other studies. The *Y. pestis* F1 antigen is the major protective component of the current human whole-cell vaccine against plague [179], although the vaccine is ineffective against $F1^-$ strains and also some $F1^+$ strains. A recombinant vaccine composed of a fusion of F1 with another protective antigen, the V antigen (an essential part of the Type III secretion system), produced specific IgG in the serum of healthy volunteers during a phase 1 trial [180]. However, no evidence for a cellular immune response was found.

A complex of recombinant SafB chaperone with SafD adhesin (both components of the ***S**almonella* **a**typical **f**imbriae, Saf) together with cholera toxin B as mucosal antigen uptake enhancer was shown to give protection in BalbC mice after oral challenge with *S. enteriditis* [181].

Goluszko et al. [182] used purified *E. coli* Dr fimbrial antigen to vaccinate C3H/HeJ mice against an experimental UTI and demonstrated reduced mortality in vaccinated animals. Kao et al. [183] have developed a synthetic-peptide consensus-sequence vaccine (Cs1) that targets the host receptor-binding domain of the Type IV pilus of *P. aeruginosa*. The vaccine provided increased protection against challenge by the four piliated strains PAK, PAO, KB7 and P1 in the A.BY/SnJ mouse model of acute *P. aeruginosa* infection.

Encouraging data have also been obtained from studies with components of the GBS pili. The combination of three pilus component proteins encoded by GBS PI-1 (GBS80 and GBS104) and PI-2a (GBS67) and a fourth conserved protein (Sip protein) provided protection to immunized mice against lethal challenge with 12 GBS strains. Protection also correlated with antigen accessibility on the bacterial surface and with the induction of opsonophagocytic antibodies [184]. Similar results were obtained with pilus component proteins of GAS. Vaccination of CD1 mice with a combination of the GAS serotype M1 pilins Cpa, Spy0128 and Spy0130 conferred $< 70\%$ protection to a lethal challenge with a mouse-adapted serotype M1 strain [163].

Conclusions

Bacterial pili provide striking examples of the sophisticated assemblies that have evolved to enable living organisms to colonise different environments. They are typically long, slender and flexible, in order to reach out to appropriate surfaces, they have adhesive properties, and they are also able to withstand physical, chemical and enzymatic stresses. The pili of pathogenic organisms are also exposed to the full scrutiny of the immune system and thus show wide antigenic variation. There are real prospects that vaccines can be developed once the full spectrum of antigenic variation is known for a particular organism, and combinations of the main variants can be used. The past few years have seen spectacular new discoveries, which have not only enhanced our understanding of how these structures are assembled, at the atomic and molecular level, but have also dramatically widened the number of species known to possess pili. Thus, pili have been found on Gram-positive pathogens such as *Streptococcus pneumoniae*, the Group A and B streptococci, and *Mycobacterium tuberculosis*, complementing the wide range of Gram-negative organisms that have long been known to express pili. These discoveries, which are profoundly important for understanding human disease, have been facilitated in large part by the availability of genome sequences; the pilin subunits tend to be encoded in gene clusters, and the ability to generate specific antibodies against the recombinant proteins has led to the detection of pili by EM, using gold-labeled antibodies. It seems remarkable that these assemblies were not seen earlier, but the explanation must lie in their extreme thinness.

Much has also been learned about pilus structure and assembly, notably in the molecular details of the donor-strand complementation mechanism, involving incomplete Ig-like domains, which is used in the chaperone-usher assembly pathway; the assembly of Type IV pilin subunits into complete pilus fibres; and most recently the first structure for a Gram-positive major pilin. A striking feature is the contrast between Gram-negative and Gram-positive pili. Those of Gram-negative organisms, typified by the Type I (or P) and Type IV pili, tend to be thicker, with their subunits associated non-covalently, and their modes of assembly give them their strength and stability. Some of these pili have other functions in addition to their adhesive roles, including roles in DNA uptake and motility, where their ability to retract is important. Gram-positive pili, on the other hand, have more passive roles that are primarily to do with cell attachment. Strikingly, the sortase-assembled pili of Gram-positive organisms are thinner and gain their strength and stability in an entirely different way, through

covalent bonds both between the pilin subunits and within them; sortase-mediated isopeptide bonds join the successive subunits of the pilus backbone (like beads on a string) and internal isopeptide bonds that form spontaneously during folding stabilise the individual subunits.

Whereas much is now known about the structure and assembly of pilus shafts, at least for the main types of pili, many questions still surround their modes of adhesion to host cells. It is generally assumed that adhesin domains reside at the pilus tip, but this is not necessarily true of all types of pili and there is much evidence that the pilus shafts also play some role in adhesion. For Gram-positive pili, for example, so-called minor pilin subunits are necessary for binding to host cells, but it is not known how or where these are attached to the shaft. Likewise, whereas some adhesin domains, such as the FimH domains of Type I pili, are known to bind host-cell glycans, for other pili the molecular targets are not known, and may involve matrix components such as collagen or fibronectin. We can expect a major focus of the next few years will be on these characterising these domains, their structures, specificities and modes of attachment, since they are indeed the "front-line troops" in the attack of pathogens on their host.

1 Anderson, T. F. (1949) In the Nature of the Bacterial Surface, Oxford University Press. Oxford, U.K.

2 Sauer, F.G., Remaut, H., Hultgren, S. J. and Waksman, G. (2004). Fiber assembly by the chaperone-usher pathway. Biochim. Biophys. Acta 1694, 259–267.

3 Capitani, G., Eidam, O., Glockshuber, R. and Grutter, M. G. (2006). Structural and functional insights into the assembly of type 1 pili from Escherichia coli. Microbes Infect 8, 2284–2290.

4 Anderson, K. L., Billington J., Pettigrew, D., Cota, E., Simpson, P., Roversi, P., Chen, H. A., Urvil, P., du Merle, L., Barlow, P. N., Medof, M. E., Smith, R. A., Nowicki, B., Le Bouguénec, C., Lea, S. M. and Matthews, S. (2004). An atomic resolution model for assembly, architecture, and function of the Dr adhesins. Mol. Cell 15, 647–657.

5 Piatek, R., Zalewska, B., Kolaj, O., Ferens, M., Nowicki, B. and Kur, J. (2005). Molecular aspects of biogenesis of Escherichia coli Dr Fimbriae: characterization of DraB-DraE complexes. Infect. Immun. 73, 135–145.

6 Zavialov, A. V., Berglund, J., Pudney, A. F., Fooks, L.J., Ibrahim, T. M., MacIntyre, S. and Knight, S. D. (2003). Structure and biogenesis of the capsular F1 antigen from Yersinia pestis: preserved folding energy drives fiber formation. Cell 113, 587–596.

7 Knight, S. D. (2007). Structure and assembly of Yersinia pestis F1 antigen. Adv. Exp. Med. Biol. 603, 74–87.

8 Thanassi, D. G., Saulino, E. T., Lombardo, M. J., Roth, R., Heuser, J. and Hultgren, S. J. (1998). The PapC usher forms an oligomeric channel: implications for pilus biogenesis across the outer membrane. Proc. Natl. Acad. Sci. U S A 95, 3146–3151.

9 Sauer, F. G., Barnhart, M., Choudhury, D., Knight, S. D., Waksman, G. and Hultgren, S. J. (2000). Chaperone-assisted pilus assembly and bacterial attachment. Curr. Opin. Struct. Biol. 10, 548–556.

10 Sauer, F. G., Mulvey, M. A., Schilling, J. D., Martinez, J. J. and Hultgren, S. J. (2000). Bacterial pili: molecular mechanisms of pathogenesis. Curr. Opin. Microbiol. 3, 65–72.

11 Sauer, F. G., Pinkner, J. S., Waksman, G. and Hultgren, S. J. (2002). Chaperone priming of pilus subunits facilitates a topological transition that drives fiber formation. Cell 111, 543–551.

12 Brinton, C. C. (1969) Contributions of pili to the specificity of the bacterial surface and a unitary hypothesis of conjugal infectious heredity. In The Specificity of Cell Surfaces (Davis, B. D. and Warren, L., ed. eds), pp. 37–70. Prentice-Hall Inc., Englewood Cliffs, NJ.

13 Hahn, E., Wild, P., Hermanns, U., Sebbel, P., Glockshuber, R., Häner, M., Taschner, N., Burkhard, P., Aebi, U. and Müller, S. A. (2002). Exploring the 3D molecular architecture of Escherichia coli type 1 pili. J. Mol. Biol. 323, 845–857.

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

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

16 Muhldorfer, I., Ziehbur, W. and Hacker, J. (2001) Escherichia coli in Urinary Tract Infections. In Molecular Medical Microbiology (Sussman, M., ed. eds), pp. 1739–1748. Academic Press, London.

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

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

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

20 Xie, B., Zhou, G., Chan, S. Y., Shapiro, E., Kong, X. P., Wu, X. R., Sun, T. T. and Costello, C. E. (2006). Distinct glycan structures of uroplakins Ia and Ib: structural basis for the selective binding of FimH adhesin to uroplakin Ia. J. Biol. Chem. 281, 14644–14653.

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

22 Sokurenko, E. V., E. V., Chesnokova, V., Dykhuizen, D. E., Ofek, I., Wu, X. R., Krogfelt, K. A., Struve, C., Schembri, M. A., and Hasty, D. L. (1998). Pathogenic adaptation of Escherichia coli by natural variation of the FimH adhesin. Proc. Natl. Acad. Sci. U S A 95, 8922–8926.

23 Bouckaert, J., Mackenzie, J., de Paz, J. L., Chipwaza, B., Choudhury, D., Zavialov, A., Mannerstedt, K., Anderson, J., Piérard, D., Wyns, L., Seeberger, P. H., Oscarson, S., De Greve, H. and Knight, S. D. (2006). The affinity of the FimH fimbrial adhesin is receptor-driven and quasi-independent of Escherichia coli pathotypes. Mol. Microbiol. 61, 1556–1568.

24 Duncan, M. J., Mann, E. L., Cohen, M. S., Ofek, I., Sharon, N. and Abraham, S. N. (2005). The distinct binding specificities exhibited by enterobacterial type 1 fimbriae are determined by their fimbrial shafts. J. Biol. Chem. 280, 37707–37716.

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

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

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

28 Eto, D. S., Jones, T. A., Sundsbak, J. L. and Mulvey, M. A. (2007). Integrin-mediated host cell invasion by type 1-piliated uropathogenic Escherichia coli. PLoS Pathog 3, e100.

29 Wright, K. J., Seed, P. C. and Hultgren, S. J. (2007). Development of intracellular bacterial communities of uropathogenic Escherichia coli depends on type 1 pili. Cell Microbiol. 9, 2230–2241.

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

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

32 Orndorff, P. E., Devapali, A., Palestrant, S., Wyse, A., Everett, M. L., Bollinger, R. R. and Parker, W. (2004). Immunoglobulin-mediated agglutination of and biofilm formation by Escherichia coli K-12 require the type 1 pilus fiber. Infect. Immun. 72, 1929–1938.

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

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

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

36 Roberts, J. A., Marklund, B. I., Ilver, D., Haslam, D., Kaack, M. B., Baskin, G., Louis, M., Möllby, R., Winberg, J. and Normark, S. (1994). The Gal(alpha 1–4)Gal-specific tip adhesin of Escherichia coli P-fimbriae is needed for pyelonephritis to occur in the normal urinary tract. Proc. Natl. Acad. Sci. U S A 91, 11889–11893.

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

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

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

40 Nowicki, B., Selvarangan, R. and Nowicki, S. (2001). Family of Escherichia coli Dr adhesins: decay-accelerating factor receptor recognition and invasiveness. J. Infect. Dis. 183 Suppl 1, S24–27.

41 Servin, A. L. (2005). Pathogenesis of Afa/Dr diffusely adhering Escherichia coli. Clin. Microbiol. Rev. 18, 264–292.

42 Korotkova, N., Cota, E., Lebedin, Y., Monpouet, S., Guignot, J., Servin, A. L., Matthews, S. and Moseley, S. L. (2006). A subfamily of Dr adhesins of Escherichia coli bind independently to decay-accelerating factor and the N-domain of carcinoembryonic antigen. J. Biol. Chem. 281, 29120–29130.

43 Goluszko, P., Selvarangan, R., Popov, V., Pham, T., Wen, J. W. and Singhal, J. (1999). Decay-accelerating factor and cytoskeleton redistribution pattern in HeLa cells infected with recombinant Escherichia coli strains expressing Dr family of adhesins. Infect. Immun. 67, 3989–3997.

44 Cota, E., Jones, C., Simpson, P., Altroff, H., Anderson, K. L., du Merle, L., Guignot, J., Servin, A., Le Bouguénec, C., Mardon, H.and Matthews, S. (2006). The solution structure of the invasive tip complex from Afa/Dr fibrils. Mol. Microbiol. 62, 356–366.

45 Diard, S., Toribio, A. L., Boum, Y., Vigier, F., Kansau, I., Bouvet, O. and Servin, A. (2006). Environmental signals implicated in Dr fimbriae release by pathogenic Escherichia coli. Microbes Infect. 8, 1851–1858.

46 Mulvey, M. A. (2002). Adhesion and entry of uropathogenic Escherichia coli. Cell Microbiol. 4, 257–271.

47 Rocha, S. P., Pelayo, J. S. and Elias, W. P. (2007). Fimbriae of uropathogenic Proteus mirabilis. FEMS Immunol. Med. Microbiol. 51, 1–7.

48 Sauer, F. G., Futterer, K., Pinkner, J. S., Dodson, K. W., Hultgren, S. J. and Waksman, G. (1999). Structural basis of chaperone function and pilus biogenesis. Science 285, 1058–1061.

49 Hung, D. L., Knight, S. D. and Hultgren, S. J. (1999). Probing conserved surfaces on PapD. Mol. Microbiol. 31, 773–783.

50 Knight, S. D., Choudhury, D., Hultgren, S., Pinkner, J., Stojanoff, V. and Thompson, A. (2002). Structure of the S pilus periplasmic chaperone SfaE at 2.2 A resolution. Acta Crystallogr. D. Biol. Crystallogr. 58, 1016–1022.

51 Krasan, G. P., Sauer, F. G., Cutter, D., Farley, M. M., Gilsdorf, J. R., Hultgren, S. J. and St Geme, J. W., 3rd. (2000). Evidence for donor strand complementation in the biogenesis of Haemophilus influenzae haemagglutinating pili. Mol. Microbiol. 35, 1335–1347.

52 Zavialov, A. V., Kersley, J., Korpela, T., Zav'yalov, V. P., MacIntyre, S. and Knight, S. D. (2002). Donor strand complementation mechanism in the biogenesis of non-pilus systems. Mol. Microbiol. 45, 983–995.

53 Poole, S. T., McVeigh, A. L., Anantha, R. P., Lee, L. H., Akay, Y. M., Pontzer, E. A., Scott, D. A., Bullitt, E.and Savarino, S.J. (2007). Donor strand complementation governs intersubunit interaction of fimbriae of the alternate chaperone pathway. Mol. Microbiol. 63, 1372–1384.

54 Zavialov, A., Zav'yalova, G., Korpela, T. and Zav'yalov, V. (2007). FGL chaperone-assembled fimbrial polyadhesins: anti-immune armament of Gram-negative bacterial pathogens. FEMS Microbiol. Rev. 31, 478–514.

55 Li, H., Qian, L., Chen, Z., Thibault, D., Liu, G., Liu, T. and Thanassi, D.G. (2004). The outer membrane usher forms a twin-pore secretion complex. J. Mol. Biol. 344, 1397–1407.

56 Nishiyama, M., Horst, R., Eidam, O., Herrmann, T., Ignatov, O., Vetsch, M., Bettendorff, P., Jelesarov, I., Grütter, M. G., Wüthrich, K., Glockshuber, R. and Capitani, G. (2005). Structural basis of chaperone-subunit complex recognition by the type 1 pilus assembly platform FimD. EMBO J 24, 2075–2086.

57 Remaut, H., Tang, C., Henderson, N. S., Pinkner, J. S., Wang, T., Hultgren, S. J., Thanassi, D. G., Waksman, G. and Li, H. (2008). Fiber formation across the bacterial outer membrane by the chaperone/usher pathway. Cell 133, 640–652.

58 Munera, D., Hultgren, S. and Fernandez, L. A. (2007). Recognition of the N-terminal lectin domain of FimH adhesin by the usher FimD is required for type 1 pilus biogenesis. Mol. Microbiol. 64, 333–346.

59 Nishiyama, M., Ishikawa, T., Rechsteiner, H. and Glockshuber, R. (2008). Reconstitution of Pilus Assembly Reveals a Bacterial Outer Membrane Catalyst. Science.

60 Ottow, J. C. (1975). Ecology, physiology, and genetics of fimbriae and pili. Annu. Rev. Microbiol. 29, 79–108.

61 Craig, L., Pique, M. E. and Tainer, J. A. (2004). Type IV pilus structure and bacterial pathogenicity. Nat. Rev. Microbiol. 2, 363–378.

62 Craig, L. and Li, J. (2008). Type IV pili: paradoxes in form and function. Curr. Opin. Struct. Biol. 18, 267–277.

63 Strom, M. S. and Lory, S. (1993). Structure-function and biogenesis of the type IV pili. Annu. Rev. Microbiol. 47, 565–596.

64 Varga, J. J., Nguyen, V., O'Brien, D. K., Rodgers, K., Walker, R. A. and Melville, S. B. (2006). Type IV pili-dependent gliding motility in the Gram-positive pathogen Clostridium

perfringens and other Clostridia. Mol. Microbiol. 62, 680–694.
65 Rakotoarivonina, H., Jubelin, G., Hebraud, M., Gaillard-Martinie, B., Forano, E. and Mosoni, P. (2002). Adhesion to cellulose of the Gram-positive bacterium Ruminococcus albus involves type IV pili. Microbiology 148, 1871–1880.
66 Bhaya, D., Bianco, N. R., Bryant, D. and Grossman, A. (2000). Type IV pilus biogenesis and motility in the cyanobacterium Synechocystis sp. PCC6803. Mol. Microbiol. 37, 941–951.
67 Mattick, J. S. (2002). Type IV pili and twitching motility. Annu. Rev. Microbiol. 56, 289–314.
68 Reguera, G., McCarthy, K. D., Mehta, T., Nicoll, J. S., Tuominen, M. T. and Lovley, D. R. (2005). Extracellular electron transfer via microbial nanowires. Nature 435, 1098–1101.
69 Wolfgang, M., van Putten, J. P., Hayes, S. F., Dorward, D. and Koomey, M. (2000). Components and dynamics of fiber formation define a ubiquitous biogenesis pathway for bacterial pili. EMBO J 19, 6408–6418.
70 Peabody, C. R., Chung, Y. J., Yen, M. R., Vidal-Ingigliardi, D., Pugsley, A. P. and Saier, M. H., Jr. (2003). Type II protein secretion and its relationship to bacterial type IV pili and archaeal flagella. Microbiology 149, 3051–3072.
71 Swanson, J. (1973). Studies on gonococcus infection. IV. Pili: their role in attachment of gonococci to tissue culture cells. J. Exp. Med. 137, 571–589.
72 Rahman, M., Kallstrom, H., Normark, S. and Jonsson, A. B. (1997). PilC of pathogenic Neisseria is associated with the bacterial cell surface. Mol. Microbiol. 25, 11–25.
73 Scheuerpflug, I., Rudel, T., Ryll, R., Pandit, J. and Meyer, T. F. (1999). Roles of PilC and PilE proteins in pilus-mediated adherence of Neisseria gonorrhoeae and Neisseria meningitidis to human erythrocytes and endothelial and epithelial cells. Infect. Immun. 67, 834–843.
74 Nassif, X., Beretti, J. L., Lowy, J., Stenberg, P., O'Gaora, P., Pfeifer, J., Normark, S. and So, M. (1994). Roles of pilin and PilC in adhesion of Neisseria meningitidis to human epithelial and endothelial cells. Proc. Natl. Acad. Sci. U S A 91, 3769–3773.
75 Kallstrom, H., Liszewski, M. K., Atkinson, J. P. and Jonsson, A. B. (1997). Membrane cofactor protein (MCP or CD46) is a cellular pilus receptor for pathogenic Neisseria. Mol. Microbiol. 25, 639–647.
76 Johansson, L., Rytkonen, A., Bergman, P., Albiger, B., Källström, H., Hökfelt, T., Agerberth, B., Cattaneo, R., and Jonsson, A. B. (2003). CD46 in meningococcal disease. Science 301, 373–375.
77 Riley-Vargas, R. C., Gill, D. B., Kemper, C., Liszewski, M. K. and Atkinson, J. P. (2004). CD46: expanding beyond complement regulation. Trends Immunol. 25, 496–503.
78 Gill, D. B., Koomey, M., Cannon, J. G. and Atkinson, J. P. (2003). Down-regulation of CD46 by piliated Neisseria gonorrhoeae. J. Exp. Med. 198, 1313–1322.
79 Lee, S. W., Bonnah, R. A., Higashi, D. L., Atkinson, J. P., Milgram, S. L. and So, M. (2002). CD46 is phosphorylated at tyrosine 354 upon infection of epithelial cells by Neisseria gonorrhoeae. J. Cell. Biol. 156, 951–957.
80 Tobiason, D. M. and Seifert, H. S. (2001). Inverse relationship between pilus-mediated gonococcal adherence and surface expression of the pilus receptor, CD46. Microbiology 147, 2333–2340.
81 Kirchner, M., Heuer, D. and Meyer, T. F. (2005). CD46-independent binding of neisserial type IV pili and the major pilus adhesin, PilC, to human epithelial cells. Infect. Immun. 73, 3072–3082.
82 Blom, A. M., Rytkonen, A., Vasquez, P., Lindahl, G., Dahlback, B. and Jonsson, A. B. (2001). A novel interaction between type IV pili of Neisseria gonorrhoeae and the human complement regulator C4B-binding protein. J. Immunol. 166, 6764–6770.
83 Winther-Larsen, H. C., Hegge, F. T., Wolfgang, M., Hayes, S. F., van Putten, J. P. and Koomey, M. (2001). Neisseria gonorrhoeae PilV, a type IV pilus-associated protein essential to human epithelial cell adherence. Proc. Natl. Acad. Sci. U S A 98, 15276–15281.
84 Hegge, F. T., Hitchen, P. G., Aas, F. E., Kristiansen, H., Løvold, C., Egge-Jacobsen, W., Panico, M., Leong, W. Y., Bull, V., Virji, M., Morris, H. R., Dell, A. and Koomey, M. (2004). Unique modifications with phosphocholine and phosphoethanolamine define alternate antigenic forms of Neisseria gonorrhoeae type IV pili. Proc. Natl. Acad. Sci. U S A 101, 10798–10803.
85 Merz, A. J., Enns, C. A. and So, M. (1999). Type IV pili of pathogenic Neisseriae elicit cortical plaque formation in epithelial cells. Mol. Microbiol. 32, 1316–1332.
86 Maier, B., Koomey, M. and Sheetz, M. P. (2004). A force-dependent switch reverses type IV pilus retraction. Proc. Natl. Acad. Sci. U S A 101, 10961–1096.
87 Merz, A. J., So, M. and Sheetz, M. P. (2000). Pilus retraction powers bacterial twitching motility. Nature 407, 98–102.
88 Pujol, C., Eugene, E., Marceau, M. and Nassif, X. (1999). The meningococcal PilT protein is required for induction of intimate attachment to epithelial cells following pilus-mediated adhesion. Proc. Natl. Acad. Sci. U S A 96, 4017–4022.
89 Wolfgang, M., Lauer, P., Park, H. S., Brossay, L., Hebert, J. and Koomey, M. (1998). PilT mutations lead to simultaneous defects in competence for natural transformation and twitching motility in piliated Neisseria gonorrhoeae. Mol. Microbiol. 29, 321–330.
90 Helaine, S., Dyer, D. H., Nassif, X., Pelicic, V. and Forest, K. T. (2007). 3D structure/function analysis of PilX reveals how minor pilins can modulate the virulence properties of type IV pili. Proc. Natl. Acad. Sci. U S A 104, 15888–15893.
91 Haas, R. and Meyer, T. F. (1986). The repertoire of silent pilus genes in Neisseria gonorrhoeae: evidence for gene conversion. Cell 44, 107–115.
92 Jonsson, A. B., Nyberg, G. and Normark, S. (1991). Phase variation of gonococcal pili by frameshift mutation in pilC, a novel gene for pilus assembly. EMBO J 10, 477–488.
93 Meyer, T. F., Gibbs, C.P. and Haas, R. (1990). Variation and control of protein expression in Neisseria. Annu. Rev. Microbiol. 44, 451–477.
94 Banerjee, A. and Ghosh, S. K. (2003). The role of pilin glycan in neisserial pathogenesis. Mol. Cell. Biochem. 253, 179–190.
95 Marceau, M., Forest, K., Beretti, J. L., Tainer, J. and Nassif, X. (1998). Consequences of the loss of O-linked glycosylation of meningococcal type IV pilin on piliation and pilus-mediated adhesion. Mol. Microbiol. 27, 705–715.
96 Chamot-Rooke, J., J., Rousseau, B., Lanternier, F., Mikaty, G., Mairey, E., Malosse, C., Bouchoux, G., Pelicic, V., Camoin, L., Nassif, X. and Duménil, G. (2007). Alternative Neisseria spp. type IV pilin glycosylation with a glyceramido acetamido trideoxyhexose residue. Proc. Natl. Acad. Sci. U S A 104, 14783–14788.
97 Hamadeh, R. M., Estabrook, M. M., Zhou, P., Jarvis, G. A. and Griffiss, J. M. (1995). Anti-Gal binds to pili of Neisseria meningitidis: the immunoglobulin A isotype blocks complement-mediated killing. Infect. Immun. 63, 4900–4906.
98 Lee, H., Hsu, F. F., Turk, J. and Groisman, E. A. (2004). The PmrA-regulated pmrC gene mediates phosphoethanolamine modification of lipid A and polymyxin resistance in Salmonella enterica. J. Bacteriol. 186, 4124–4133.
99 Ram, S., S., Cox, A. D., Wright, J. C., Vogel, U., Getzlaff, S., Boden, R., Li, J., Plested, J. S., Meri, S., Gulati, S., Stein, D. C., Richards, J. C., Moxon, E. R.and Rice, P. A. (2003). Neisserial lipooligosaccharide is a target for complement component C4b. Inner core phosphoethanolamine residues define C4b linkage specificity. J. Biol. Chem. 278, 50853–50862.
100 Weiser, J. N., Pan, N., McGowan, K. L., Musher, D., Martin, A. and Richards, J. (1998). Phosphorylcholine on the lipopolysaccharide of Haemophilus influenzae contributes to persistence in the respiratory tract and sensitivity to serum killing mediated by C-reactive protein. J. Exp. Med. 187, 631–640.

101 Cundell, D. R., Gerard, N. P., Gerard, C., Idanpaan-Heikkila, I. and Tuomanen, E. I. (1995). Streptococcus pneumoniae anchor to activated human cells by the receptor for platelet-activating factor. Nature 377, 435–438.

102 Parge, H. E., Forest, K. T., Hickey, M. J., Christensen, D. A., Getzoff, E. D. and Tainer, J. A. (1995). Structure of the fibre-forming protein pilin at 2.6 A resolution. Nature 378, 32–38.

103 Craig, L., Volkmann, N., Arvai, A. S., Pique, M. E., Yeager, M., Egelman, E. H. and Tainer, J. A. (2006). Type IV pilus structure by cryo-electron microscopy and crystallography: implications for pilus assembly and functions. Mol. Cell. 23, 651–662.

104 Mutlu, G. M. and Wunderink, R. G. (2006). Severe pseudomonal infections. Curr. Opin. Crit. Care 12, 458–463.

105 Folkhard, W., Marvin, D. A., Watts, T. H. and Paranchych, W. (1981). Structure of polar pili from Pseudomonas aeruginosa strains K and O. J. Mol. Biol. 149, 79–93.

106 Suh, J. Y., Spyracopoulos, L., Keizer, D. W., Irvin, R. T. and Sykes, B. D. (2001). Backbone dynamics of receptor binding and antigenic regions of a Pseudomonas aeruginosa pilin monomer. Biochemistry 40, 3985–3995.

107 Lee, K. K., Sheth, H. B., Wong, W. Y., Sherburne, R., Paranchych, W., Hodges, R. S., Lingwood, C. A., Krivan, H., and Irvin, R. T. (1994). The binding of Pseudomonas aeruginosa pili to glycosphingolipids is a tip-associated event involving the C-terminal region of the structural pilin subunit. Mol. Microbiol. 11, 705–713.

108 Ramphal, R., Sadoff, J. C., Pyle, M. and Silipigni, J. D. (1984). Role of pili in the adherence of Pseudomonas aeruginosa to injured tracheal epithelium. Infect. Immun. 44, 38–40.

109 Chi, E., Mehl, T., Nunn, D. and Lory, S. (1991). Interaction of Pseudomonas aeruginosa with A549 pneumocyte cells. Infect Immun 59, 822–828.

110 Castric, P., Cassels, F. J. and Carlson, R. W. (2001). Structural characterization of the Pseudomonas aeruginosa 1244 pilin glycan. J. Biol. Chem. 276, 26479–26485.

111 Voisin, S., Kus, J. V., Houliston, S., St-Michael, F., Watson, D., Cvitkovitch, D. G., Kelly, J., Brisson ,J.R.and Burrows, L.L. (2007). Glycosylation of Pseudomonas aeruginosa strain Pa5196 type IV pilins with mycobacterium-like alpha-1,5-linked d-Araf oligosaccharides. J. Bacteriol. 189, 151–159.

112 Nataro, J. P. and Kaper, J. B. (1998). Diarrheagenic Escherichia coli. Clin. Microbiol. Rev. 11, 142–201.

113 Giron, J. A., Ho, A. S. and Schoolnik, G. K. (1991). An inducible bundle-forming pilus of enteropathogenic Escherichia coli. Science 254, 710–713.

114 Knutton, S., Shaw, R. K., Anantha, R. P., Donnenberg, M. S. and Zorgani, A. A. (1999). The type IV bundle-forming pilus of enteropathogenic Escherichia coli undergoes dramatic alterations in structure associated with bacterial adherence, aggregation and dispersal. Mol. Microbiol. 33, 499–509.

115 Jagannatha, H. M., Sharma, U. K., Ramaseshan, T., Surolia, A. and Balganesh, T. S. (1991). Identification of carbohydrate structures as receptors for localised adherent enteropathogenic Escherichia coli. Microb. Pathog. 11, 259–268.

116 Scaletsky, I. C., Milani, S. R., Trabulsi, L. R. and Travassos, L. R. (1988). Isolation and characterization of the localized adherence factor of enteropathogenic Escherichia coli. Infect. Immun. 56, 2979–2983.

117 Vanmaele, R. P., Heerze, L. D. and Armstrong, G. D. (1999). Role of lactosyl glycan sequences in inhibiting enteropathogenic Escherichia coli attachment. Infect. Immun. 67, 3302–3307.

118 Hyland, R. M., Sun, J., Griener, T. P., Mulvey, G. L., Klassen, J. S., Donnenberg, M. S. and Armstrong, G. D. (2008). The bundlin pilin protein of enteropathogenic Escherichia coli is an N-acetyllactosamine-specific lectin. Cell. Microbiol. 10, 177–187.

119 Barnett Foster, D., Philpott, D., Abul-Milh, M., Huesca, M., Sherman, P. M. and Lingwood, C. A. (1999). Phosphatidylethanolamine recognition promotes enteropathogenic E. coli and enterohemorrhagic E. coli host cell attachment. Microb. Pathog. 27, 289–301.

120 Hicks, S., Frankel, G., Kaper, J. B., Dougan, G. and Phillips, A. D. (1998). Role of intimin and bundle-forming pili in enteropathogenic Escherichia coli adhesion to pediatric intestinal tissue in vitro. Infect. Immun. 66, 1570–1578.

121 Ramboarina, S., Fernandes, P. J., Daniell, S., Islam, S., Simpson, P., Frankel, G., Booy, F., Donnenberg, M. S. and Matthews, S. (2005). Structure of the bundle-forming pilus from enteropathogenic Escherichia coli. J. Biol. Chem. 280, 40252–40260.

122 Tacket, C. O., Taylor, R. K., Losonsky, G., Lim, Y., Nataro, J. P., Kaper, J. B. and Levine, M. M. (1998). Investigation of the roles of toxin-coregulated pili and mannose-sensitive hemagglutinin pili in the pathogenesis of Vibrio cholerae O139 infection. Infect. Immun. 66, 692–295.

123 Craig, L., Taylor, R. K., Pique, M. E., Adair, B. D., Arvai, A. S., Singh, M., Lloyd, S. J., Shin, D. S., Getzoff, E. D., Yeager, M., Forest, K. T., and Tainer, J. A. (2003). Type IV pilin structure and assembly: X-ray and EM analyses of Vibrio cholerae toxin-coregulated pilus and Pseudomonas aeruginosa PAK pilin. Mol. Cell. 11, 1139–1150.

124 Li, J., Lim, M. S., Li, S., Brock, M., Pique, M. E., Woods, V. L., Jr. and Craig, L. (2008). Vibrio cholerae toxin-coregulated pilus structure analyzed by hydrogen/deuterium exchange mass spectrometry. Structure 16, 137–148.

125 Karaolis, D. K., Johnson, J. A., Bailey, C. C., Boedeker, E. C., Kaper, J. B. and Reeves, P. R. (1998). A Vibrio cholerae pathogenicity island associated with epidemic and pandemic strains. Proc. Natl. Acad. Sci. U S A 95, 3134–3139.

126 Waldor, M. K. and Mekalanos, J. J. (1996). Lysogenic conversion by a filamentous phage encoding cholera toxin. Science 272, 1910–1914.

127 Peek, J. A. and Taylor, R. K. (1992). Characterization of a periplasmic thiol:disulfide interchange protein required for the functional maturation of secreted virulence factors of Vibrio cholerae. Proc. Natl. Acad. Sci. U S A 89, 6210–6214.

128 Strom, M. S., Nunn, D. N. and Lory, S. (1993). A single bifunctional enzyme, PilD, catalyzes cleavage and N-methylation of proteins belonging to the type IV pilin family. Proc. Natl. Acad. Sci. U S A 90, 2404–2408.

129 Collins, R. F., Saleem, M. and Derrick, J. P. (2007). Purification and three-dimensional electron microscopy structure of the Neisseria meningitidis type IV pilus biogenesis protein PilG. J. Bacteriol. 189, 6389–6396.

130 Crowther, L. J., Anantha, R. P. and Donnenberg, M. S. (2004). The inner membrane subassembly of the enteropathogenic Escherichia coli bundle-forming pilus machine. Mol. Microbiol. 52, 67–79.

131 Burrows, L. L. (2005). Weapons of mass retraction. Mol. Microbiol. 57, 878–88.

132 Satyshur, K. A., Worzalla, G. A., Meyer, L. S., Heiniger, E. K., Aukema, K. G., Misic, A. M. and Forest, K. T. (2007). Crystal structures of the pilus retraction motor PilT suggest large domain movements and subunit cooperation drive motility. Structure 15, 363–376.

133 Bayan, N., Guilvout, I. and Pugsley, A. P. (2006). Secretins take shape. Mol. Microbiol. 60, 1–4.

134 Collins, R. F., Frye, S. A., Kitmitto, A., Ford, R. C., Tonjum, T. and Derrick, J. P. (2004). Structure of the Neisseria meningitidis outer membrane PilQ secretin complex at 12 A resolution. J. Biol. Chem. 279, 39750–39756.

135 Olsen, A., Jonsson, A. and Normark, S. (1989). Fibronectin binding mediated by a novel class of surface organelles on Escherichia coli. Nature 338, 652–625.

136 Chapman, M. R., Robinson, L. S., Pinkner, J. S., Roth, R., Heuser, J., Hammar, M., Normark, S. and Hultgren, S. J. (2002). Role of Escherichia coli curli operons in directing amyloid fiber formation. Science 295, 851–855.

137 Collinson, S. K., Parker, J. M., Hodges, R. S. and Kay, W. W. (1999). Structural predictions of AgfA, the insoluble fimbrial

subunit of Salmonella thin aggregative fimbriae. J. Mol. Biol. 290, 741–756.
138 Austin, J. W., Sanders, G., Kay, W. W. and Collinson, S. K. (1998). Thin aggregative fimbriae enhance Salmonella enteritidis biofilm formation. FEMS Microbiol. Lett. 162, 295–301.
139 Kikuchi, T., Mizunoe, Y., Takade, A., Naito, S. and Yoshida, S. (2005). Curli fibers are required for development of biofilm architecture in Escherichia coli K-12 and enhance bacterial adherence to human uroepithelial cells. Microbiol. Immunol. 49, 875–884.
140 Herwald, H., Morgelin, M., Olsen, A., Rhen, M., Dahlback, B., Muller-Esterl, W. and Bjorck, L. (1998). Activation of the contact-phase system on bacterial surfaces–a clue to serious complications in infectious diseases. Nat. Med. 4, 298–302.
141 Tukel, C., Raffatellu, M., Humphries, A. D., Wilson, R. P., Andrews-Polymenis, H. L., Gull, T., Figueiredo, J. F., Wong, M. H., Michelsen, K. S., Akçelik, M., Adams, L. G.and Bäumler, A. J. (2005). CsgA is a pathogen-associated molecular pattern of Salmonella enterica serotype Typhimurium that is recognized by Toll-like receptor 2. Mol. Microbiol. 58, 289–304.
142 Bian, Z. and Normark, S. (1997). Nucleator function of CsgB for the assembly of adhesive surface organelles in Escherichia coli. EMBO J. 16, 5827–5836.
143 Sakellaris, H. and Scott, J. R. (1998). New tools in an old trade: CS1 pilus morphogenesis. Mol. Microbiol. 30, 681–687.
144 Qadri, F., Svennerholm, A. M., Faruque, A. S. and Sack, R. B. (2005). Enterotoxigenic Escherichia coli in developing countries: epidemiology, microbiology, clinical features, treatment, and prevention. Clin. Microbiol. Rev. 18, 465–483.
145 Perez-Casal, J., Swartley, J. S. and Scott, J. R. (1990). Gene encoding the major subunit of CS1 pili of human enterotoxigenic Escherichia coli. Infect. Immun. 58, 3594–3600.
146 Sakellaris, H., Munson, G. P. and Scott, J. R. (1999). A conserved residue in the tip proteins of CS1 and CFA/I pili of enterotoxigenic Escherichia coli that is essential for adherence. Proc. Natl. Acad. Sci. U S A 96, 12828–12832.
147 Froehlich, B. J., Karakashian, A., Melsen, L. R., Wakefield, J. C. and Scott, J. R. (1994). CooC and CooD are required for assembly of CS1 pili. Mol. Microbiol. 12, 387–401.
148 Voegele, K., Sakellaris, H. and Scott, J. R. (1997). CooB plays a chaperone-like role for the proteins involved in formation of CS1 pili of enterotoxigenic Escherichia coli. Proc. Natl. Acad. Sci. U S A 94, 13257–13261.
149 Yanagawa, R., Otsuki, K. and Tokui, T. (1968). Electron microscopy of fine structure of Corynebacterium renale with special reference to pili. Jpn J Vet Res 16, 31–37.
150 Ton-That, H. and Schneewind, O. (2004). Assembly of pili in Gram-positive bacteria. Trends Microbiol. 12, 228–234.
151 Telford, J. L., Barocchi, M. A., Margarit, I., Rappuoli, R. and Grandi, G. (2006). Pili in gram-positive pathogens. Nat. Rev. Microbiol. 4, 509–519.
152 Ton-That, H. and Schneewind, O. (2003). Assembly of pili on the surface of Corynebacterium diphtheriae. Mol. Microbiol. 50, 1429–1438.
153 Marraffini, L. A., Dedent, A. C. and Schneewind, O. (2006). Sortases and the art of anchoring proteins to the envelopes of gram-positive bacteria. Microbiol. Mol. Biol. Rev. 70, 192–221.
154 Hadfield, T. L., McEvoy, P., Polotsky, Y., Tzinserling, V. A. and Yakovlev, A. A. (2000). The pathology of diphtheria. J. Infect. Dis. 181 Suppl. 1, S116–120.
155 Ton-That, H., Marraffini, L. A. and Schneewind, O. (2004). Sortases and pilin elements involved in pilus assembly of Corynebacterium diphtheriae. Mol Microbiol 53, 251–261.
156 Mandlik, A., Swierczynski, A., Das, A. and Ton-That, H. (2007). Corynebacterium diphtheriae employs specific minor pilins to target human pharyngeal epithelial cells. Mol. Microbiol. 64, 111–124.
157 Doran, K. S. and Nizet, V. (2004). Molecular pathogenesis of neonatal group B streptococcal infection: no longer in its infancy. Mol. Microbiol. 54, 23–31.
158 Lauer, P., Rinaudo, C. D., Soriani, M., Margarit, I., Maione, D., Rosini, R., Taddei, A. R., Mora, M., Rappuoli, R., Grandi, G.and Telford, J. L. (2005). Genome analysis reveals pili in Group B Streptococcus. Science 309, 105.
159 Rosini, R., Rinaudo, C. D., Soriani, M., Lauer, P., Mora, M., Maione, D., Taddei, A., Santi, I., Ghezzo, C., Brettoni, C., Buccato, S., Margarit, I., Grandi, G. and Telford, J. L. (2006). Identification of novel genomic islands coding for antigenic pilus-like structures in Streptococcus agalactiae. Mol. Microbiol. 61, 126–141.
160 Dramsi, S., Caliot, E., Bonne, I., Guadagnini, S., Prévost, M.C., Kojadinovic, M., Lalioui, L., Poyart, C.and Trieu-Cuot, P. (2006). Assembly and role of pili in group B streptococci. Mol. Microbiol. 60, 1401–1413.
161 Krishnan, V., Gaspar, A. H., Ye, N., Mandlik, A., Ton-That, H. and Narayana, S. V. (2007). An IgG-like domain in the minor pilin GBS52 of Streptococcus agalactiae mediates lung epithelial cell adhesion. Structure 15, 893–903.
162 Cunningham, M. W. (2008). Pathogenesis of group A streptococcal infections and their sequelae. Adv. Exp. Med. Biol. 609, 29–42.
163 Mora, M., Bensi, G., Capo, S., Falugi, F., Zingaretti, C., Manetti, A. G., Maggi, T., Taddei, A. R., Grandi, G.and Telford, J. L. (2005). Group A Streptococcus produce pilus-like structures containing protective antigens and Lancefield T antigens. Proc. Natl. Acad. Sci. U S A 102, 15641–15646.
164 Bessen, D. E. and Kalia, A. (2002). Genomic localization of a T serotype locus to a recombinatorial zone encoding extracellular matrix-binding proteins in Streptococcus pyogenes. Infect. Immun. 70, 1159–1167.
165 Kang, H. J., Coulibaly, F., Clow, F., Proft, T. and Baker, E. N. (2007). Stabilizing isopeptide bonds revealed in gram-positive bacterial pilus structure. Science 318, 1625–1628.
166 Manetti, A. G., Zingaretti, C., Falugi, F., Capo, S., Bombaci, M., Bagnoli, F., Gambellini, G., Bensi, G., Mora, M., Edwards, A. M., Musser, J. M., Graviss, E. A., Telford, J. L., Grandi, G. and Margarit, I. (2007). Streptococcus pyogenes pili promote pharyngeal cell adhesion and biofilm formation. Mol. Microbiol. 64, 968–983.
167 Abbot, E. L., Smith, W. D., Siou, G. P., Chiriboga, C., Smith, R. J., Wilson, J. A., Hirst, B.H. and Kehoe, M. A. (2007). Pili mediate specific adhesion of Streptococcus pyogenes to human tonsil and skin. Cell Microbiol 9, 1822–1833.
168 Edwards, A. M., Manetti, A. G., Falugi, F., Zingaretti, C., Capo, S., Buccato, S., Bensi, G., Telford, J. L., Margarit, I., and Grandi, G. (2008). Scavenger receptor gp340 aggregates group A streptococci by binding pili. Mol. Microbiol. 68, 1378–1394.
169 Kadioglu, A., Weiser, J. N., Paton, J. C. and Andrew, P. W. (2008). The role of Streptococcus pneumoniae virulence factors in host respiratory colonization and disease. Nat. Rev. Microbiol 6, 288–301.
170 LeMieux, J., Hava, D. L., Basset, A. and Camilli, A. (2006). RrgA and RrgB are components of a multisubunit pilus encoded by the Streptococcus pneumoniae rlrA pathogenicity islet. Infect. Immun. 74, 2453–2456.
171 Barocchi, M. A., Ries, J., Zogaj, X., Hemsley, C., Albiger, B., Kanth, A., Dahlberg, S., Fernebro, J., Moschioni, M., Masignani, V., Hultenby, K., Taddei, A. R., Beiter, K., Wartha, F., von Euler, A., Covacci, A., Holden, D. W., Normark, S., Rappuoli, R. and Henriques-Normark, B. (2006). A pneumococcal pilus influences virulence and host inflammatory responses. Proc. Natl. Acad. Sci. U S A 103, 2857–2862.
172 Hilleringmann, M., Giusti, F., Baudner, B. C., Masignani, V., Covacci, A., Rappuoli, R., Barocchi, M. A. and Ferlenghi, I. (2008). Pneumococcal pili are composed of protofilaments exposing adhesive clusters of Rrg A. PLoS. Pathog. 4, e1000026.
173 Mishra, A., Das, A., Cisar, J. O. and Ton-That, H. (2007). Sortase-catalyzed assembly of distinct heteromeric fimbriae in Actinomyces naeslundii. J. Bacteriol 189, 3156–3165.

174 Gibbons, R. J., Hay, D. I., Cisar, J. O. and Clark, W. B. (1988). Adsorbed salivary proline-rich protein 1 and statherin: receptors for type 1 fimbriae of Actinomyces viscosus T14V-J1 on apatitic surfaces. Infect. Immun. 56, 2990–2903.
175 Mergenhagen, S. E., Sandberg, A. L., Chassy, B. M., Brennan, M. J., Yeung, M. K., Donkersloot, J.A. and Cisar, J.O. (1987). Molecular basis of bacterial adhesion in the oral cavity. Rev. Infect. Dis. 9 Suppl. 5, S467–274.
176 Navarre, W. W. and Schneewind, O. (1999). Surface proteins of gram-positive bacteria and mechanisms of their targeting to the cell wall envelope. Microbiol. Mol. Biol. Rev. 63, 174–229.
177 Swaminathan, A., Mandlik, A., Swierczynski, A., Gaspar, A., Das, A. and Ton-That, H. (2007). Housekeeping sortase facilitates the cell wall anchoring of pilus polymers in Corynebacterium diphtheriae. Mol. Microbiol. 66, 961–974.
178 Brinton, C. C. (1978) Immunobiology of Neisseria gonnorhoeae, American Society for Microbiology. Washington DC.
179 Li, B., Jiang, L., Song, Q., Yang, J., Chen, Z., Guo, Z., Zhou, D., Du, Z., Song, Y., Wang, J., Wang, H., Yu, S., Wang, J., and Yang, R. (2005). Protein microarray for profiling antibody responses to Yersinia pestis live vaccine. Infect. Immun. 73, 3734–3739.
180 Williamson, E. D., Flick-Smith, H. C., Lebutt, C., Rowland, C. A., Jones, S. M., Waters, E. L., Gwyther, R. J., Miller, J., Packer, P. J. and Irving, M. (2005). Human immune response to a plague vaccine comprising recombinant F1 and V antigens. Infect. Immun. 73, 3598–3608.
181 Strindelius, L., Filler, M. and Sjoholm, I. (2004). Mucosal immunization with purified flagellin from Salmonella induces systemic and mucosal immune responses in C3H/HeJ mice. Vaccine 22, 3797–3808.
182 Goluszko, P., Goluszko, E., Nowicki, B., Nowicki, S., Popov, V. and Wang, H. Q. (2005). Vaccination with purified Dr Fimbriae reduces mortality associated with chronic urinary tract infection due to Escherichia coli bearing Dr adhesin. Infect. Immun. 73, 627–631.
183 Kao, D. J., Churchill, M. E., Irvin, R. T. and Hodges, R. S. (2007). Animal protection and structural studies of a consensus sequence vaccine targeting the receptor binding domain of the type IV pilus of Pseudomonas aeruginosa. J. Mol. Biol. 374, 426–442.
184 Maione, D., Margarit, I., Rinaudo, C. D., Masignani, V., Mora, M., Scarselli, M., Tettelin, H., Brettoni, C., Iacobini, E. T., Rosini, R., D'Agostino, N., Miorin, L., Buccato, S., Mariani, M., Galli, G., Nogarotto, R., Nardi Dei, V., Vegni, F., Fraser, C., Mancuso, G., Teti, G., Madoff, L. C., Paoletti, L. C., Rappuoli, R., Kasper, D. L., Telford, J. L. and Grandi, G. (2005). Identification of a universal Group B streptococcus vaccine by multiple genome screen. Science 309, 148–150.