THE
EMBO
JOURNAL

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New EMBO Member's Review

Architectures and biogenesis of non-flagellar protein appendages in Gram-negative bacteria

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Bacteria commonly expose non-flagellar proteinaceous appendages on their outer surfaces. These extracellular structures, called pili or fimbriae, are employed in attachment and invasion, biofilm formation, cell motility or protein and DNA transport across membranes. Over the past 15 years, the power of molecular and structural techniques has revolutionized our understanding of the biogenesis, structure, function and mode of action of these bacterial organelles. Here, we review the five known classes of Gram-negative non-flagellar appendages from a biosynthetic and structural point of view.

Introduction

Anderson (1949) and Houwink (1949) were the first to notice the presence of non-flagellar appendages on the outer surface of bacteria. Early reports on bacterial appendages produced a confusing and varied nomenclature including 'bristles', 'cilia', 'filaments', 'fimbriae', 'fibrillae', 'pili' or 'needles' (Duguid and Anderson, 1967). Over the years, however, the terms 'pili' (Latin, hairs—hair-like structures) and 'fimbriae' (Latin, threads) have most frequently been used, whereas the terms 'filaments' and 'needles' now designate appendages associated with two subclasses of type III secretion apparatus (Cornelis, 2006). In the post-genomic era, it has now become clear that known Gram-negative non-flagellar appendages are part of five major classes based on their biosynthetic path-

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way: chaperone–usher (CU) pili, curli, type IV pili, type III secretion needle and type IV secretion pili (Figure 1).

CU pili/fimbriae

The CU pathway is responsible for the synthesis of linear multisubunit pili/fimbriae mainly found in Enterobacteriaceae and in a number of *Pseudomonas*, *Haemophilus*, *Bordetella*, *Xylella*, *Burkholderia*, *Acinetobacter* and *Ralstonia* species (Sauer *et al*, 2004). As more bacterial genomes are sequenced, it has become clear that CU pili form the most abundant group of bacterial cell surface appendages. CU pili often constitute important virulence factors, responsible for specific host attachment and/or the evasion of host responses (Wright *et al*, 2007; Zavialov *et al*, 2007).

Classification and nomenclature of CU fimbriae

An early hierarchical classification of CU systems based on conserved structural elements in the chaperones identified two distinct subgroups, FGL- and FGS-chaperone assembled pili (FGL and FGS standing for '$\underline{F}_1\underline{G}_1$-long' and '$\underline{F}_1\underline{G}_1$-short', respectively (see details below)), which correspond with the assembly of thin fibrillar and rod-like pili, respectively (Hung *et al*, 1996). However, recent phylogenetic analysis of usher sequences in all 189 known CU systems revealed that only the FGL-assembled organelles form a monophyletic group, with FGS-assembled organelles composed of several diverse phylogenetic clades (Nuccio and Baumler, 2007). CU systems are now divided into 6 major clades: α-, β-, γ- (subdivided into γ_1, γ_2, γ_3 and γ_4), κ-, π- and σ-fimbriae, based on common usher ancestry, and supported by similarities in operon structure and morphology of organelles within the separate clades (Nuccio and Baumler, 2007).

CU pilus morphology and structure

CU-assembled organelles are formed by linear, unbranched polymers of several hundreds to thousands of pilus subunits, also called pilins, that range in size from $\sim$12 to $\sim$20 kDa. CU organelles differ widely in complexity and morphology, ranging from thin fibrillar material to thick fimbriae composed of a helically wound rod and a distinct fibrillar tip structure.

Fibrillar organelles are formed by CU systems belonging to the κ (*Escherichia coli* K88- and K99-related fimbriae) and γ_3 (FGL-chaperone-assembled organelles) fimbrial clades (Nuccio and Baumler, 2007). γ_3-Fimbriae are simple in architecture, containing just a single polymerizing subunit, possibly
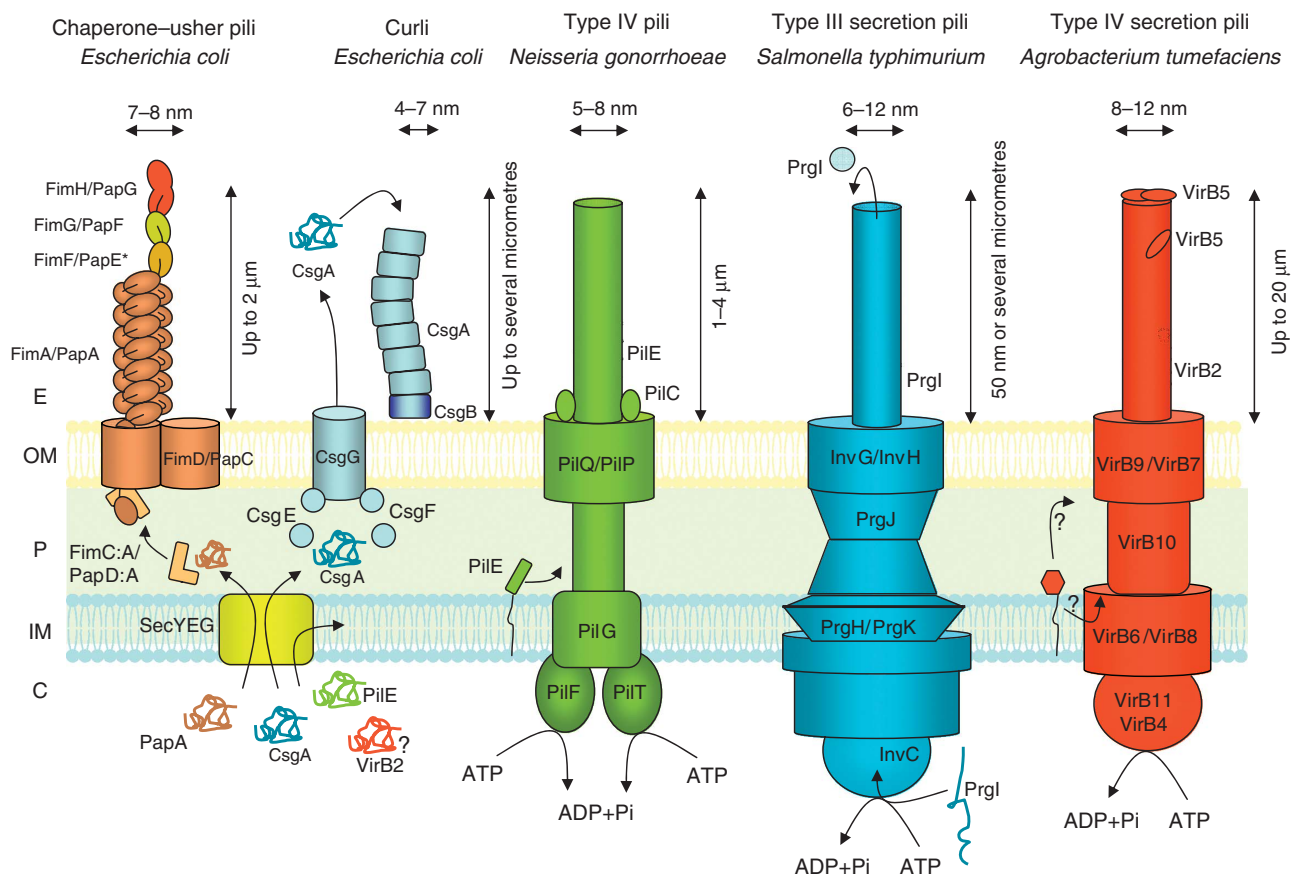
Figure 1 Pili and their assembly machineries in Gram-negative bacteria. Schematic view of the different pili found at the surface of Gram-negative bacteria. Chaperone–usher (CU) pili and curli are fibres attached on the cell surface. For the CU pili, both the P and type 1 pilus subunits and assembly proteins are shown. * indicates (1) that there are 5–10 PapE subunits in the tip fibrillum of the P pilus, whereas the equivalent subunit in the type 1 pilus system, FimF, is present in only one copy; (2) that the PapK adaptor between the PapE and PapA polymers does not have an equivalent in type 1 pili. CU pili and curli are assembled by simple systems located at the outer membrane. Type IV pili, type III secretion and type IV secretion pili are assembled by large multisubunit complexes crossing the whole bacterial cell envelope.

accompanied by a non-polymerizing subunit that caps the fibres at their distal end (Zavialov *et al*, 2007). At least in the case of *E. coli* Afa/Dr adhesins, this additional, non-polymerizing subunit serves as an invasin, inducing uptake of the bacteria by the host cell (Garcia *et al*, 2000). FGL or γ_3 systems assemble into thin flexible fibres of ~ 2–3 nm thickness (often referred to as fibrillae) that frequently curl up into a capsule-like amorphous mass on the bacterial surface (Keller *et al*, 2002). κ-Fimbrial systems contain 3–5 pilus subunit types, which assemble into thin flexible fibrillae of 2–5 nm thickness that extend individually from the bacterial surface (Hahn *et al*, 2000; Nuccio and Baumler, 2007).

Rod-like, or 'typical', fimbrial organelles are found in the α-, γ- and π-fimbrial clades, with UPEC P and type 1 pili (π- and γ_1-fimbrial clades, respectively) and CS1 fimbriae in enterotoxigenic *E. coli* (ETEC; α-fimbrial clade) as prototypical examples. These CU systems typically assemble 7- to 10-nm-thick rigid pili with an often remarkably complex quaternary organization made up of multiple subunit types. P pili, for example, comprise no less than six different subunit types, arranged into two distinct subassemblies, a flexible distal tip fibrillum of ~ 2 nm diameter, displayed onto a long, rigid 6.8-nm-wide pilus rod (Figure 1) (Kuehn *et al*, 1992). The P pilus rod is formed by a homopolymer of > 1000 copies of the PapA subunit. The PapA homopolymer organizes into a

right-handed, one-start helical structure with a 2.5 nm pitch and 3.3 subunits per turn. The P pilus tip fibrillum contains a specialized adhesive subunit, PapG, present in a single copy located at the distal end of the pilus and displayed onto a short extended and flexible polymer of 5–10 PapE subunits. Two additional subunit types, PapF and PapK, serve as single-copy linker subunits between the tip adhesin and the PapE polymer and the PapE polymer and the PapA rod, respectively. Finally, the sixth pilus subunit, PapH, serves as terminator subunit, halting pilus growth at the basal end by arresting PapA polymerization (Verger *et al*, 2006). Type 1 pili (γ_1-fimbrial clade) display a very similar architecture, but form shorter tip fibrillae (Figure 1) (Hahn *et al*, 2002). α-Fimbrial organelles (alternate CU pathway) such as ETEC CS1 and CFA/I fimbriae lack a visible tip fibrillum all together and here the adhesive subunit is connected directly to the polymerizing major pilus subunit (Evans *et al*, 1979; Levine *et al*, 1984). No functional or structural information is available for σ- and β-fimbriae: these families were defined entirely based on usher sequences and the fimbriae associated with these systems have not yet been observed.

CU pilus assembly

The CU biosynthetic pathway forms a terminal branch of the general secretory pathway and involves just two proprietary
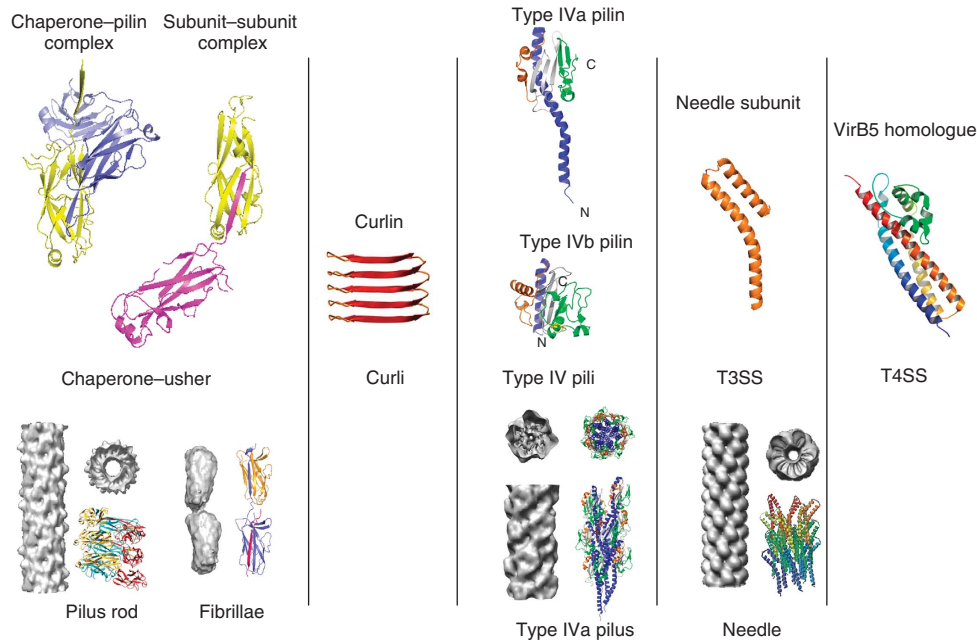
Figure 2 Pilins and pili structures. *Chaperone–usher pathway*: (top) crystal structure of PapD–PapA chaperone–subunit and PapA–PapA subunit–subunit complexes (Verger *et al*, 2007). (Bottom) Rod: cryo-EM density map (Mu and Bullitt, 2006) and model of the PapA rod (Sauer *et al*, 1999). (Bottom) Fibrillae: negative stain EM map (left) and model (right) of the SafA fibre (Salih *et al*, 2008). *Curli*: hypothetical CsgA and CsgB structure model (Barnhart and Chapman, 2006). *Type IV pili*: (top) structure of the *N. gonorrhoeae* GC pilin (type IVa pilin) (Craig *et al*, 2006) and *V. cholerae* TcpA pilin (type IVb pilin) (Craig *et al*, 2003). (Bottom) Cryo-EM density map of the GC pilus at 12.5 Å (left) and structural model of the pilin assembly within the pilus (right) (Craig *et al*, 2006). *Type III secretion pili*: (top) *Shigella flexneri* needle subunit MxiH crystal structure (Deane *et al*, 2006). (Bottom) cryo-EM density map of the *S. flexneri* needle and structural model of the pilin within the pilus (Deane *et al*, 2006). *Type IV secretion pili*: crystal structure of pKM101 TraC (VirB5 homologue) (Yeo *et al*, 2003).

assembly proteins: a specialized periplasmic chaperone and outer membrane assembly platform, called the usher (Sauer *et al*, 2004). Nascent pilus subunits cross the inner membrane via the Sec translocase from where they travel to the outer membrane usher as binary chaperone–subunit complexes. The chaperone aids subunit folding in the periplasm and maintains subunits in a polymerization-prone folding state (Barnhart *et al*, 2000; Sauer *et al*, 2002; Zavialov *et al*, 2003; Vetsch *et al*, 2004). The periplasmic chaperones are formed of two Ig-like domains that meet at a right angle (Holmgren and Bränden, 1989). CU pilus subunits are characterized by a non-canonical immunoglobulin fold, lacking the C-terminal β-strand (Figure 2) (Choudhury *et al*, 1999; Sauer *et al*, 1999) and by an unstructured N-terminal sequence termed 'N-terminal extension (Nte)'. The missing strand leaves a deep groove that runs along the entire subunit structure. In the chaperone–subunit complexes, the chaperone inserts part of a β-strand (strand G_1) in the subunit's groove and thus complements *in trans* the incomplete fold of the pilus subunit, a process called donor-strand complementation (Figure 3A, top panel) (Choudhury *et al*, 1999; Sauer *et al*, 1999). During subunit polymerization, the complementing β-strand donated by the chaperone is replaced by the Nte peptide of a newly incoming subunit, a process called donor-strand exchange (DSE) (Figure 3A, top panel) (Sauer *et al*, 2002; Zavialov *et al*, 2003; Remaut *et al*, 2006).

The DSE reaction proceeds through a zip-in-zip-out mechanism, whereby the process of DSE is initiated by the insertion of a residue (the so-called 'P5 residue') in the incoming subunit's Nte into a binding pocket (the so-called

'P5 pocket') located within the receiving subunit's groove. This initial binding event is followed by the zippering-in of the incoming Nte within the receiving groove, whereas the G_1 strand of the chaperone zips out (Remaut *et al*, 2006) (Figure 3A, bottom panel). As DSE progresses, the receiving subunit structure transitioned from a chaperone-stabilized high-energy semi-unfolded state to a lower energy folded state (Sauer *et al*, 2002; Zavialov *et al*, 2003). Remarkably, pilus biogenesis is an ATP-independent process. It is believed that it is the folding energy released upon DSE that drives subunit polymerization. Incorporation of the subunit PapH in the growing pilus terminates pilus biogenesis. This is because PapH is the only subunit deprived of a P5 pocket and, consequently, is unable to undergo DSE (Verger *et al*, 2006).

Subunit polymerization occurs at the OM usher. The usher recruits chaperone–subunit complexes to the outer membrane, catalyses their ordered polymerization and is responsible for pilus translocation to the outer surface (Nishiyama *et al*, 2008; Remaut *et al*, 2008). OM ushers are ∼800 residue proteins comprised of four functional domains. The N-terminal ∼125 residues form a distinct soluble periplasmic domain that binds chaperone–subunit complexes (Ng *et al*, 2004; Nishiyama *et al*, 2005). A second periplasmic domain is formed by the C-terminal ∼150 residues. Though essential, the exact role of this domain is unclear. The central part of the protein gives rise to the usher translocation channel. This consists of a 24-stranded β-pore with an approximate inner diameter of 45 Å × 25 Å, compatible with passage of folded pilus subunits (Remaut *et al*, 2008) (Figure 3B). This β-barrel domain is interrupted at strands 6 and 7 by a soluble
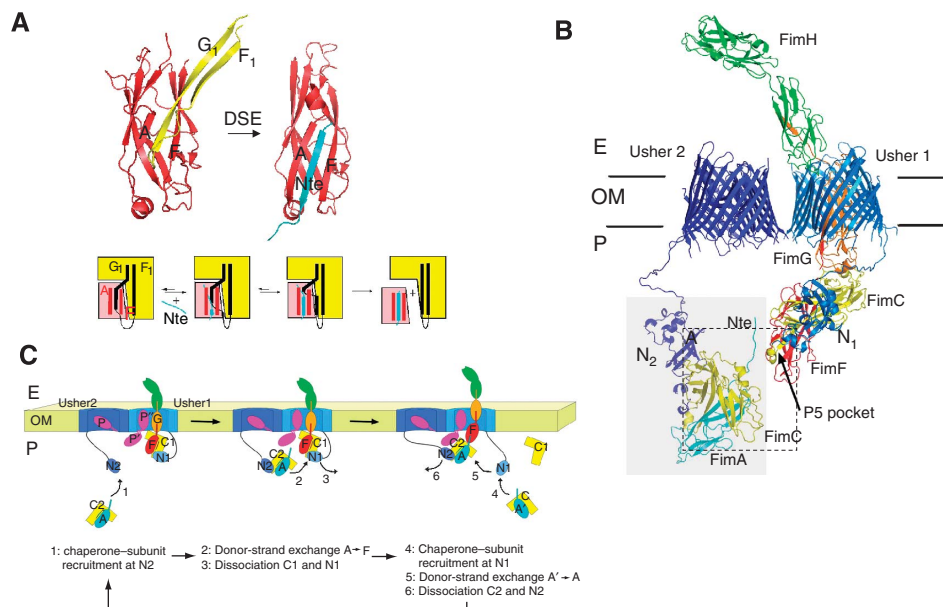
Figure 3 Model of type 1 pilus assembly at the OM usher. The tip fibrillum of type 1 pili is composed of three single-copy subunits: FimH, the adhesin, and the FimG and FimF subunits. FimA is the major subunit of the type 1 pilus and is assembled immediately after FimF. In the type 1 pilus system, the chaperone is encoded by the *fimC* gene, whereas the usher is encoded by the *fimD* gene. Expression of the *fimC*, *fimD*, *fimF*, *fimG* and *fimH* genes results in the assembly of a $FimD_2$:C:F:G:H complex where FimH is in donor-strand exchange with FimG, itself in donor-strand exchange with FimF. FimF is also in donor-strand complementation with FimC. In that complex, two protomers of FimD are present (Remaut *et al*, 2008). (**A**) The donor-strand complementation and donor-strand exchange mechanisms. (Top left) The subunit in red is shown in donor-strand complementation with the chaperone G_1 strand in yellow. For clarity, only the F_1 and G_1 strands of the chaperone are shown. The A and F strands of the subunit are labelled. (Top right) The same subunit in red is shown in donor-strand exchange with the incoming subunit Nte (in cyan). For clarity, only the Nte peptide of that subunit is shown. (Bottom) Schematic representation of the concerted zip-in-zip-out donor-strand exchange mechanism. The diagrams show the F_1-G_1 β-hairpin (black) in the chaperone (yellow) and the A and F strands in the subunit (in red). The attacking Nte peptide is in cyan. (**B**) The type 1 $FimD_2$:C:F:G:H complex in the process of recruiting a FimC:FimA complex. The model shows the $FimD_2$:C:F:G:H complex as derived by cryo-EM (Remaut *et al*, 2008) coloured blue, dark blue, yellow, red orange and green for FimD usher pore 1, 2, the FimC chaperone, FimF, FimG and the FimH adhesin, respectively. In addition, the model shows the tentative position (shaded in light grey) of an incoming FimC:A complex bound to usher 2's N-terminal domain ($FimD_{N2}$:C:A, coloured dark blue, yellow, cyan, respectively, and labelled N2, FimC and FimA, respectively). N1 indicates the N-terminal domain of usher 1. The dashed rectangle labelled 'A' refers to the region, the zoomed-in representation of which is shown in (A). (**C**) Schematic diagram of pilus assembly. The diagram shows the usher twinned pores (in blue and dark blue for usher 1 and 2, respectively), the ushers' N-terminal domains (N1 and N2 for usher 1 and 2 in blue and dark blue, respectively), the FimH adhesin (H; in green), FimG (G; in orange), FimC:F (C1:F; in yellow and red) and an incoming FimC:A complex (C2:A; in yellow and cyan). The plug domain is in magenta. As usher 2 is not used for secretion, the plug (P) remains in place obstructing the usher 2 pore. In the activated usher 1, two alternative positions are proposed (P′ and P″): the plug could either move sideways (P′) or be ejected from the pore (P″). Note that the mechanism for pore activation or gating is unknown but is likely to be triggered by the binding of the chaperone–adhesin complex (Nishiyama *et al*, 2008; Remaut *et al*, 2008). For clarity, the C-terminal domains of usher 1 and 2 are not shown. At left, the $FimD_2$:C:F:G:H complex (as represented in B) recruits an incoming FimC:A complex through binding to the N-terminal domain of usher 2 (N2; step 1). The complex is brought within donor-strand exchange of FimF, resulting in the release of the FimF-bound chaperone (C1) and the dissociation of the N-terminal domain of usher 1 (N1) (middle panel; steps 2 and 3). N1 is now free to recruit another FimC:A complex (labelled C:A′; right panel, step 4), and bring the complex within proximity of the N2-bound FimC:A complex (step 5). Donor-strand exchange then releases N2 for recruitment of the next chaperone–subunit complex (step 6). Iteration of alternating binding to released usher N-terminal domains, followed by donor-strand exchange with the penultimate chaperone–subunit complex leads to stepwise growth of the pilus fibre (steps 1 through 6).

β-sandwich 'plug' domain ($\sim$110 residues) that blocks the channel in its non-activated form. OM ushers function as homodimeric twinned-pore complexes (Li *et al*, 2004; So and Thanassi, 2006; Remaut *et al*, 2008). The cryo-EM reconstruction of a three-subunit assembly intermediate of the UPEC type 1 pilus shows that, remarkably, just a single usher pore is used for pilus translocation (Figure 3B) (Remaut *et al*, 2008). The requirement for the second usher copy to make a functional assembly complex is believed to stem from the need for two chaperone–subunit recruitment sites to allow polymerization at the periplasmic side of the membrane. In this model, both usher protomers alternatively recruit the incoming chaperone–subunit complexes to the assembly platform through their N-terminal domain (see details in Figure 3C) (Remaut *et al*, 2008).

Curli

Curli are a fairly recently identified class of bacterial filaments expressed on the outer surfaces of Enterobacteriaceae (Olsen *et al*, 1989; Barnhart and Chapman, 2006). In *Escherichia* and *Salmonella* species, they form abundant, highly aggregative flexible fibres of $\sim$4–7 nm diameter called 'thin aggregative fimbriae' that form the major proteinaceous component of the extracellular matrix and together with cellulose, are associated with a distinct multicellular morphotype on Congo red plates known as rdar (red, dry and rough) (Zogaj *et al*, 2001; Chapman *et al*, 2002).

Curli has a role in bacterial pathogenesis by promoting cell adhesion and invasion, and studies in mice reveal curli fibres can enhance amyloidosis in the host by acting as a cross-

seeding nuclei (Gophna *et al*, 2001; Lundmark, 2005). Curli are also potent promoters of a proinflammatory response and can enhance hypotension and bleeding disorders during sepsis and septic chock by their absorption of contact-phase proteins and fibrinogen (Herwald *et al*, 1998).

Curli structure and assembly

The genes involved in curli production are organized into two adjacent divergently transcribed operons, *csgBAC/agfBAC* and *csgDEFG/agfDEFG* in *E. coli/Salmonella enterica*, respectively (Hammar *et al*, 1995; Collinson *et al*, 1996). Curli biogenesis is proposed to follow an extracellular nucleation-precipitation pathway that involves aggregation of the secreted major subunit (CsgA) onto a membrane-associated minor subunit (CsgB) that exerts an effect as a nucleation factor (Figure 1) (Hammar *et al*, 1996; Chapman *et al*, 2002). Subunit secretion across the outer membrane is dependent on a pore-forming outer membrane lipoprotein (CsgG) (Robinson *et al*, 2006). Fibre assembly further requires two periplasmic accessory proteins (CsgE and CsgF), both of which can physically interact with the outer membrane partner CsgG (Robinson *et al*, 2006). The exact role of these periplasmic components is ill understood. CsgE appears to be required for the secretion of the major curli subunit CsgA, whereas CsgF influences the ability of secreted CsgA to aggregate onto the bacterial surface, suggesting a role in maturation or localization of the nucleator subunit, CsgB (Chapman *et al*, 2002; Gibson *et al*, 2007). Recent data in *Salmonella* suggest CsgC (AgfC) is also involved in the assembly process as a periplasmic factor, with CsgC knockout mutants influencing the structural features of aggregated CsgA (Gibson *et al*, 2007). CsgD does not form part of the assembly machinery but functions as a transcriptional regulator (Hammar *et al*, 1995).

Both curli subunits are ~100 residues in size and each contains five repeating sequence motifs, including conserved glycines, glutamines and asparagines, commonly observed in amyloidogenic peptides. Computer modelling of CsgA and CsgB predicts a compact cross-β domain composed of five stacking β-hairpins (Figure 2) (White *et al*, 2001; Barnhart and Chapman, 2006). Curli are unbranched filaments likely formed by the edge-to-edge aggregation of these cross-β domains along the length of the fibre.

Type IV pili

Type IV pili are pilin polymers produced by many Gram-negative bacteria, including major human pathogens such as *Neisseria gonorrhoeae*, *N. meningitidis*, *Pseudomonas aeruginosa*, *Vibrio cholerae*, *S. enterica*, *Legionella pneumophila* and enteropathogenic *E. coli* (EPEC). Type IV pili are long (1–4 μm) and flexible filaments of 5–8 nm in diameter that can resist stress forces greater than 100 pN (Merz *et al*, 2000; Maier *et al*, 2002). Some bacteria such as *V. cholerae* and EPEC express bundled type IV pili (Craig *et al*, 2003; Ramboarina *et al*, 2005). These pili are essential for a large number of processes determining bacterial virulence, including auto-aggregation, adhesion, twitching motility, biofilm formation or cellular invasion (Craig *et al*, 2004; Burrows, 2005).

Classification of the pilins and maturation: type IVa and IVb pilins

Type IV pilins are produced by rather evolutionarily divergent Gram-negative bacteria. However, these proteins have been grouped in the same class because they share common sequence features and are post-translationally modified in the same manner. Indeed, they are all produced as precursors that possess a basic N-terminal leader sequence. Then signal peptide removal and N-methylation are performed by a single bi-functional endopeptidase first identified in *P. aeruginosa* (PilD), which cleaves between an invariant glycine residue and a phenylalanine (or rarely methionine) (Nunn and Lory, 1991). All the type IV pilins contain a 30 amino-acid hydrophobic N terminus with an invariant glutamic acid residue at the fifth position of the mature polypeptide. In addition, the C-terminal part of the pilin contains two conserved cystein residues. Type IVa and IVb pilins differ by the length of the signal peptide: type IVa pilin has a short 5–6 leader peptide, whereas type IVb pilins contain a longer one (15–30 amino acids).

Pilus structure

For clarity, we chose to only present in this review the latest full-length type IVa *N. gonorrhoeae* GC and N-terminally truncated type IVb *V. cholerae* TcpA structures as representative examples of their respective families (Craig *et al*, 2003, 2006).

Type IVa and IVb pilins share conserved structural features: a conserved structural core (Figure 2 in grey) flanked by a conserved extended N-terminal α1 helix (Figure 2 in blue), which is itself flanked by two variable regions commonly called αβ-loop and D-region (in orange and green in Figure 2, respectively). The fold of the core domain is different in type IVa and IVb pilins: whereas the GC pilin displays four β-strands that are directly inter-connected, the TcpA pilin shows a more complex connectivity and contains a fifth β-strand. The two variable regions, α/β and D, are exposed on both sides of the molecule. The α/β region shows great structural variations among the type IV pilins. In addition, O-glycosylation and phosphorylation occurs in the GC pilin.

The structural model of *N. gonorrhoeae* GC pilus recently proposed by Craig *et al* was deduced from a 12.5-Å resolution cryo-EM density map of the native pilus (Figure 2). In this model, the pilus has an outer diameter of 60 Å and a narrow central channel between 6 and 11 Å. The crystal structure of a single native GC pilin was docked into the EM map. The α1-helices of the pilin interact tightly with each other in a three helices bundle to form the hydrophobic core of the pilus within the filament. The globular heads are pointing outwards exposing the variable α/β and D-regions to the surface of the pilus. In addition, the α/β and D-regions are also involved in pilin–pilin interactions within the filament. The overall type IVb pili architecture is very similar to that of type IVa pili (Craig *et al*, 2003).

Assembly–disassembly

Unlike curli and CU pili, type IV pilus assembly requires a large assembly machinery that is ATP driven and spans both inner and outer membranes. Quite remarkably, and unique among the different bacterial cell surface filaments, type IV pili assembly can be reversed and the formed pili retracted.

Several bacterial processes such as twitching motility (Merz *et al*, 2000) or DNA uptake (Aas *et al*, 2002) rely specifically on this pilus disassembly mechanism. The assembly–disassembly mechanism requires the presence of conserved ATPases called PilB/F (*P. aeruginosa/N. gonorrhoeae* nomenclature) and PilT (Figure 1) (Turner *et al*, 1993). The PilB/F ATPase would be required for pilus growth, whereas PilT would catalyse a rapid depolymerization mechanism (1500 subunits/s) (Burrows, 2005). These two ATPases are homologous to the 'traffic ATPases' found in type II and IV secretion systems. In the *Aquiflex aeolicus* PilT structure (Satyshur *et al*, 2007), it was proposed that a 15 Å movement of the C-terminal domain observed upon nucleotide hydrolysis and γ-phosphate release would be sufficient to provide the mechanical force necessary to extract pilin subunits from the pilus during disassembly.

The pilus is likely to be assembled at the inner membrane from a mature pilin 'pool' that is inserted into the inner membrane through the N-terminal hydrophobic part of the α1-helix (Strom and Lory, 1987). The cytosolic ATPase PilB is thought to be recruited to the pilus base through an inner membrane-embedded protein (possibly PilC/G; Figure 1; Crowther *et al*, 2004). At the molecular level, the assembly–disassembly mechanism is far from elucidated, and present assembly–disassembly molecular models are highly speculative (see details in Craig *et al*, 2006).

PilQ forms a ∼1 MDa dodecameric structure that forms the outer membrane pore that assists the pilus across the outer membrane (Figure 1) (Wolfgang *et al*, 2000; Collins *et al*, 2005). This protein is part of the outer membrane secretins family, which comprises secretins used in type II and III secretion or filamentous phage release mechanisms (Marlovits *et al*, 2004; Chami *et al*, 2005). PilQ interacts with the pilotin lipoprotein PilP (Hardie *et al*, 1996; Drake *et al*, 1997; Balasingham *et al*, 2007). The pilotin proteins have been shown to be important in the stabilization, the oligomerization and/or the outer membrane localization of many secretins.

Type III secretion-related appendage

Type III secretion-related appendages were first observed in *S. typhimurium* as needle-like surface structures responsible for bacterial entry into cultured epithelial cells and as a pilus-like structure in *P. syringae* and other plant pathogens (called the Hrp pilus) (Roine *et al*, 1997; Kubori *et al*, 1998). The assembly of these structures was dependent on systems now well established to form a flagellum-like secretion nanomachine known as the type III secretion system (T3SS). T3SS assembles a complex injectisome designed to secrete effector proteins across the bacterial and host cell envelopes (Cornelis, 2006). These secretory devices are found in a range of animal and plant pathogens including Enterobacteriaceae as well as *Aeromonas*, *Burkholderia*, *Chlamydia*, *Chromobacterium*, *Pseudomonas*, *Ralstonia*, *Vibrio* and *Xanthomonas* species. So far, no adhesive role has been observed for T3SS appendages.

Needle structure and assembly

Injectisome assembly involves over 20 proteins (Cornelis, 2006). The injectisome basal structure forms a large cylindrical heterocomplex with two double rings that span the inner

and outer membranes and that are linked by a hollow structure that crosses the intermediate periplasmic space (Figure 1) (Kubori *et al*, 1998). At its cytoplasmic side, the basal body is contacted by ATPases and accessory proteins responsible for driving and ordering protein secretion and filament assembly (Woestyn *et al*, 1994; Akeda and Galan, 2005; Muller *et al*, 2006). On the outer surface, the basal body assembles a hollow filamentous structure.

The basic extracellular structure, as first identified in the *Salmonella* SPI-1 T3SS (Kubori *et al*, 1998) and later observed in detail from purified *Shigella* injectisomes (Tamano *et al*, 2000; Blocker *et al*, 2001), is a short rigid hollow 'needle' of about 60 nm in length and inner diameter of 2–3 nm. The basic needle is composed of a homopolymer of 100–150 ∼9 kDa subunits (PrgJ, MxiH, YscF and Escf in *Salmonella*, *Shigella*, *Yersinia* and EPEC, respectively). These subunits reveal a helix-loop-helix structure similar to the D0 portion of flagellin (Figure 2) (Deane *et al*, 2006). Similar to flagellin, needle subunits polymerize into a hollow superhelical structure. The distal end of the needle carries a distinct tip complex composed of the protein LcrV (*Yersinia*), one of the three translocators involved in pore formation in the target cytoplasmic membrane (Mueller *et al*, 2005). LcrV forms a dumbbell-shaped structure that has been modelled as a pentameric complex at the tip of the needle and thought of as providing the scaffolding base for the additional two translocator proteins, assembled upon contact with the target membrane (YopB and YopD in *Yersinia*) (Derewenda *et al*, 2004; Deane *et al*, 2006). In EPEC, this needle base is extended by a longer flexible 'filament' up to 600 nm in length (Knutton *et al*, 1998; Sekiya *et al*, 2001) and composed of an EspA homopolymer. In plant pathogens, the needles are replaced altogether by a long flexible 'pilus', the Hrp pilus (Roine *et al*, 1997; Li *et al*, 2002).

Assembly of T3SS appendages is thought to follow a mechanism similar to that seen in flagella (He *et al*, 2004; Macnab, 2004). The components that form the base complex are assembled mostly in a Sec-dependent manner. Filament subunits and secretion substrates, however, lack classical N-terminal leader sequences and are secreted through the nascent secretion apparatus itself. In flagella, the extracellular hook and flagellar filament are formed by the secretion of subunits directly from the cytoplasm through the base complex and through the growing hollow filament (Macnab, 2004). In T3SS filaments, growth by subunit secretion at the distal end has been directly demonstrated in the case of the Hrp pilus and the EspA filament in *P. syringae* and EPEC injectisomes, respectively (Li *et al*, 2002; Crepin *et al*, 2005). The inner dimensions of the flagella and T3SS filaments argue that filament subunits and T3SS effectors traverse the structure in a largely extended, unfolded state.

In *Yersinia*, the length of the needle complex is linearly correlated with the primary structure of the YscP protein, therefore also called a 'molecular ruler' (Journet *et al*, 2003). A hypothetical mechanism has been proposed in which the molecular ruler resides inside the needle and allows secretion of needle subunits until the needle reaches the length of the unfolded, extended ruler protein. In the *Salmonella* SPI-1 T3SS, a different mechanism for needle length control has been suggested (Marlovits *et al*, 2006). Purified *Salmonella* injectisomes indicate the presence of an inner rod structure (possibly composed of a specialized subunit, PrgJ) inside the

basal body of the secretion system. In the proposed model, secretion of rod and needle subunits (PrgJ and PrgI, respectively; Figure 1) occurs simultaneously. Completion of the inner rod assembly signals a halt in secretion of rod and needle subunits, thereby controlling needle length (Marlovits et al, 2006).

Type IV secretion pili

Type IV secretion systems (T4SSs) are ancestrally derived from the mating pair formation cluster proteins involved in bacterial conjugation. T4SSs are also used by plant and human pathogens such as *Agrobacterium tumefaciens*, *Bordetella pertussis*, *Legionella pneumonia* and *Helicobacter pylori* to secrete virulence factors (DNA and/or proteins) into host cells. T4SS pili are thought to establish a stable and specific contact between cells before substrate transfer (Schroder and Lanka, 2005).

Morphology

T4SS pili are classified into two major groups: IncF-like pili (conjugative pili produced by Inc-F, -H, -T and -J systems) and IncP-like pili (conjugative pili produced by Inc-P, -N, -W systems) (Lawley et al, 2003) The IncF-like pili are long (2–20 μm) and flexible appendages with 8–9 nm in diameter; IncP-like pili are short (<1 μm) and rigid rods with 8–12 nm in diameter (Bradley, 1980). These different pili types are assembled by two different classes of conjugative T4SSs: IncP-type and IncF-type T4SSs. IncP-type T4SSs are type IVa secretion systems. They are composed of VirB-like components (in reference to the *A. tumefaciens* VirB/D4 T4SS where 11 VirB proteins (VirB1-11) and 1 VirD proteins (VirD4) are essential for T4SS structure, assembly and function (Figure 1)). IncF-type T4SSs are type IVb secretion systems: they not only contain VirB-like components but also additional proteins that do not have any counterpart in IncP-type T4SSs.

In the case of T4SSs that are not involved in bacterial conjugation, the morphology of the pilus is rarely known or they cannot be classified into the subgroups defined above. For example, the *A. tumefaciens* T-pilus has a different morphology (T-pili are variable in length and flexible with 10 nm in diameter) than IncP-like and IncF-like pili even if the VirB/D4 T4SS organization is very similar to IncP conjugative T4SSs (Eisenbrandt et al, 1999). *H. pylori* produces 100–200 nm needle-like type IV secretion-related appendages at the tip of which CagA (a *H. pylori* T4SS substrate) can be found (Kwok et al, 2007).

Pilus structure

Fibre X-ray diffraction and electron microscopy studies of the F-pilus showed that these pili are cylindrical filaments with an external diameter of 8 nm and an internal lumen of 2 nm in diameter. These filaments exhibit a 5-fold helical symmetry (Marvin and Folkhard, 1986).

In *A. tumefaciens*, it has been shown that VirB5 and VirB7 are minor T-pilus components in addition to the major structural pilin VirB2 (Schmidt-Eisenlohr et al, 1999; Lai and Kado, 2000; Sagulenko et al, 2001b). These proteins have homologues in other pathogenic bacteria such as *Brucella suis* or *Bartonella henselae* (called VirB5/VirB7 in both species) and also in conjugative plasmids such as pKM101 (TraC/TraN),

RP4 (TrbF/TrbH), R388 (TrwJ/TrwH) or F factor (TraE/TraV). The atomic structure of VirB5 homologue of pKM101 conjugative plasmid (called TraC) is composed of a three long helices bundle flanked by a smaller globular part (Figure 2) (Yeo et al, 2003). It has been suggested that VirB5 homologues may have adhesive functions (Schmidt-Eisenlohr et al, 1999; Yeo et al, 2003).

Pilus assembly

Little is known concerning the assembly of the type IV secretion pili. Before being assembled in the pilus, the pre-pilin undergoes several conserved steps of maturation that can be categorized into two classes.

After signal peptide removal, most of the IncF-like pilins are inserted into the inner membrane and N-acetylated. In the case of the F-pilus, the pilin TraA is N-acetylated by TraX (Moore et al, 1993). TraQ is an inner membrane chaperone-like protein, which is essential for the correct insertion and accumulation of the pilin in the inner membrane (Lu et al, 2002).

The IncP-like pilins such as conjugative RP4 TrbC and *A. tumefaciens* VirB2 are cyclic peptides (Eisenbrandt et al, 1999). In TrbC, the pre-pilin is 145 residues long and undergoes a first maturation event where its last 27 amino acids are cleaved by an unidentified endopeptidase. Then, the 36 amino-acid N-terminal signal peptide is removed by LepB. Finally, the plasmid-encoded TraF is responsible for TrbC cyclization (Kalkum et al, 2002).

Pilus assembly is clearly dependent on the integrity of the T4SS. T4SSs, where each component of the T4SS has been mutated, fail to assemble a pilus (Fullner et al, 1996; Haase et al, 1995). Pilus production and substrate transfer functions in T4SSs are clearly distinct: mutations in some T4SS components or pilins have been shown to uncouple pilus biogenesis and substrate transfer (Eisenbrandt et al, 1999; Sagulenko et al, 2001a; Jakubowski et al, 2004).

Concluding remarks

Gram-negative bacteria assemble a wide range of extracellular appendages on their outer surfaces, which they use to interact with their environment. Functionally, non-flagellar appendages appear to have specialized roles either in adhesion or in transport. CU pili, type IV pili or curli help pathogenic bacteria to specifically recognize and adhere or even invade their target cells and are not involved in transport. T3SS appendages are used as transport devices and have no described adhesive properties, whereas T4SS pili could be the only pili to have dual roles. However, non-flagellar appendages can be viewed as functionally complementary. Indeed, bacteria using secretion systems to inject virulence factors generally rely on adhesive molecules such as CU pili, type IV pili or other adhesive proteins (e.g. autotransporter and TPS adhesins) to specifically target their host cells (see for example, *B. pertussis* (Jacob-Dubuisson et al, 1994).

Bacteria have developed sophisticated ways to select, attach and infect their target cells. Adhesive and secretion pili are key protagonists during these events. Further progress in structural biology of bacterial pili will help to understand how they are assembled and how they are involved in pathogenicity. This will open avenues for interference with their function as virulence factors, or for their use as display and/or delivery tools in future nanobiotechnology applications.

References

Aas FE, Wolfgang M, Frye S, Dunham S, Lovold C, Koomey M (2002) Competence for natural transformation in *Neisseria gonorrhoeae*: components of DNA binding and uptake linked to type IV pilus expression. *Mol Microbiol* **46:** 749–760

Akeda Y, Galan JE (2005) Chaperone release and unfolding of substrates in type III secretion. *Nature* **437:** 911–915

Anderson TF (1949) In *The Nature of the Bacterial Surface*, Miles AA, Pirie NW (eds), p 76 Oxford: Oxford University Press

Balasingham SV, Collins RF, Assalkhou R, Homberset H, Frye SA, Derrick JP, Tonjum T (2007) Interactions between the lipoprotein PilP and the secretin PilQ in *Neisseria meningitidis. J Bacteriol* **189:** 5716–5727

Barnhart MM, Chapman MR (2006) Curli biogenesis and function. *Annu Rev Microbiol* **60:** 131–147

Barnhart MM, Pinkner JS, Soto GE, Sauer FG, Langermann S, Waksman G, Frieden C, Hultgren SJ (2000) PapD-like chaperones provide the missing information for folding of pilin proteins. *Proc Natl Acad Sci USA* **97:** 7709–7714

Blocker A, Jouihri N, Larquet E, Gounon P, Ebel F, Parsot C, Sansonetti P, Allaoui A (2001) Structure and composition of the *Shigella flexneri* 'needle complex', a part of its type III secretion. *Mol Microbiol* **39:** 652–663

Bradley DE (1980) Morphological and serological relationships of conjugative pili. *Plasmid* **4:** 155–169

Burrows LL (2005) Weapons of mass retraction. *Mol Microbiol* **57:** 878–888

Chami M, Guilvout I, Gregorini M, Remigy HW, Muller SA, Valerio M, Engel A, Pugsley AP, Bayan N (2005) Structural insights into the secretin PulD and its trypsin-resistant core. *J Biol Chem* **280:** 37732–37741

Chapman MR, Robinson LS, Pinkner JS, Roth R, Heuser J, Hammar M, Normark S, Hultgren SJ (2002) Role of *Escherichia coli* curli operons in directing amyloid fiber formation. *Science* **295:** 851–855

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

Collins RF, Frye SA, Balasingham S, Ford RC, Tonjum T, Derrick JP (2005) Interaction with type IV pili induces structural changes in the bacterial outer membrane secretin PilQ. *J Biol Chem* **280:** 18923–18930

Collinson SK, Clouthier SC, Doran JL, Banser PA (1996) *Salmonella enteritidis* agfBAC operon encoding thin, aggregative fimbriae. *J Bacteriol* **178:** 662–667

Cornelis GR (2006) The type III secretion injectisome. *Nat Rev Microbiol* **4:** 811–825

Craig L, Pique ME, Tainer JA (2004) Type IV pilus structure and bacterial pathogenicity. *Nat Rev Microbiol* **2:** 363–378

Craig L, Taylor RK, Pique ME, Adair BD, Arvai AS, Singh M, Lloyd SJ, Shin DS, Getzoff ED, Yeager M, Forest KT, Tainer JA (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

Craig L, Volkmann N, Arvai AS, Pique ME, Yeager M, Egelman EH, Tainer JA (2006) Type IV pilus structure by cryo-electron microscopy and crystallography: implications for pilus assembly and functions. *Mol Cell* **23:** 651–662

Crepin VF, Shaw R, Abe CM, Knutton S, Frankel G (2005) Polarity of enteropathogenic *Escherichia coli* EspA filament assembly and protein secretion. *J Bacteriol* **187:** 2881–2889

Crowther LJ, Anantha RP, Donnenberg MS (2004) The inner membrane subassembly of the enteropathogenic *Escherichia coli* bundle-forming pilus machine. *Mol Microbiol* **52:** 67–79

Deane JE, Roversi P, Cordes FS, Johnson S, Kenjale R, Daniell S, Booy F, Picking WD, Picking WL, Blocker AJ, Lea SM (2006) Molecular model of a type III secretion system needle: implications for host-cell sensing. *Proc Natl Acad Sci USA* **103:** 12529–12533

Derewenda U, Mateja A, Devedjiev Y, Routzahn KM, Evdokimov AG, Derewenda ZS, Waugh DS (2004) The structure of *Yersinia pestis* V-antigen, an essential virulence factor and mediator of immunity against plague. *Structure* **12:** 301–306

Drake SL, Sandstedt SA, Koomey M (1997) PilP, a pilus biogenesis lipoprotein in *Neisseria gonorrhoeae*, affects expression of PilQ as a high-molecular-mass multimer. *Mol Microbiol* **23:** 657–668

Duguid JP, Anderson ES (1967) Terminology of bacterial fimbriae, or pili, and their types. *Nature* **215:** 89–90

Eisenbrandt R, Kalkum M, Lai EM, Lurz R, Kado CI, Lanka E (1999) Conjugative pili of IncP plasmids, and the Ti plasmid T pilus are composed of cyclic subunits. *J Biol Chem* **274:** 22548–22555

Evans DG, Evans DJ, Clegg S, Pauley JA (1979) Purification and characterization of the CFA/I antigen of enterotoxigenic *Escherichia coli. Infect Immun* **25:** 738–748

Fullner KJ, Lara JC, Nester EW (1996) Pilus assembly by *Agrobacterium* T-DNA transfer genes. *Science* **273:** 1107–1109

Garcia MI, Jouve M, Nataro JP, Gounon P, Le Bouguénec C (2000) Characterization of the AfaD-like family of invasins encoded by pathogenic *Escherichia coli. FEBS Lett* **479:** 111–117

Gibson DL, White AP, Rajotte CM, Kay WW (2007) AgfC and AgfE facilitate extracellular thin aggregative fimbriae synthesis in *Salmonella enteritidis. Microbiology* **153:** 1131–1140

Gophna U, Barlev M, Seijffers R, Oelschlager TA, Hacker J, Ron EZ (2001) Curli fibers mediate internalization of *Escherichia coli* by eukaryotic cells. *Infect Immun* **69:** 2659–2665

Haase J, Lurz R, Grahn AM, Bamford DH, Lanka E (1995) Bacterial conjugation mediated by plasmid RP4: RSF1010 mobilization, donor-specific phage propagation, and pilus production require the same Tra2 core components of a proposed DNA transport complex. *J Bacteriol* **177:** 4779–4791

Hahn E, Wild P, Hermanns U, Sebbel P, Glockshuber R, Haner M, Taschner N, Burkhard P, Aebi U, Muller SA (2002) Exploring the 3D molecular architecture of *Escherichia coli* type 1 pili. *J Mol Biol* **323:** 845–857

Hahn E, Wild P, Schraner EM, Bertschinger HU, Häner M, Müller SA, Aebi U (2000) Structural analysis of F18 fimbriae expressed by porcine toxigenic *Escherichia coli. J Struct Biol* **132:** 241–250

Hammar M, Arnqvist A, Bian Z, Olsén A, Normark S (1995) Expression of two csg operons is required for production of fibronectin- and Congo red-binding curli polymers in *Escherichia coli* K-12. *Mol Microbiol* **18:** 661–670

Hammar M, Bian Z, Normark S (1996) Nucleator-dependent intercellular assembly of adhesive curli organelles in *Escherichia coli. Proc Natl Acad Sci USA* **93:** 6562–6566

Hardie KR, Lory S, Pugsley AP (1996) Insertion of an outer membrane protein in *Escherichia coli* requires a chaperone-like protein. *EMBO J* **15:** 978–988

He S, Nomura K, Whittam T (2004) Type III protein secretion mechanism in mammalian and plant pathogens. *Biochim Biophys Acta Mol Cell Res* **1694:** 181–206

Herwald H, Moergelin M, Olsen A, Rhen M (1998) Activation of the contact-phase system on bacterial surfaces—a clue to serious complications in infectious diseases. *Nat Med* **4:** 298–302

Holmgren A, Brändén CI (1989) Crystal structure of chaperone protein PapD reveals an immunoglobulin fold. *Nature* **342:** 248–251

Houwink AL (1949) In *The Nature of the Bacterial Surface*, Miles AA, Pirie NW (eds), p 92 Oxford: Oxford University Press

Hung DL, Knight SD, Woods RM, Pinkner JS, Hultgren SJ (1996) Molecular basis of two subfamilies of immunoglobulin-like chaperones. *EMBO J* **15:** 3792–3805

Jacob-Dubuisson F, Striker R, Hultgren SJ (1994) Chaperone-assisted self-assembly of pili independent of cellular energy. *J Biol Chem* **269:** 12447–12455

Jakubowski SJ, Krishnamoorthy V, Cascales E, Christie PJ (2004) *Agrobacterium tumefaciens* VirB6 domains direct the ordered export of a DNA substrate through a type IV secretion system. *J Mol Biol* **341:** 961–977

Journet L, Agrain C, Broz P, Cornelis GR (2003) The needle length of bacterial injectisomes is determined by a molecular ruler. *Science* **302:** 1757–1760

Kalkum M, Eisenbrandt R, Lurz R, Lanka E (2002) Tying rings for sex. *Trends Microbiol* **10:** 382–387

Keller R, Ordonez JG, de Oliveira RR, Trabulsi LR, Baldwin TJ, Knutton S (2002) Afa, a diffuse adherence fibrillar adhesin associated with enteropathogenic *Escherichia coli. Infect Immun* **70:** 2681–2689

Knutton S, Rosenshine I, Pallen MJ, Nisan I, Neves BC, Bain C, Wolff C, Dougan G, Frankel G (1998) A novel EspA-associated surface organelle of enteropathogenic *Escherichia coli* involved in protein translocation into epithelial cells. *EMBO J* **17:** 2166–2176

Kubori T, Matsushima Y, Nakamura D, Uralil J, Lara-Tejero M, Sukhan A, Galan JE, Aizawa SI (1998) Supramolecular structure of the *Salmonella typhimurium* type III protein secretion system. *Science* **280:** 602–605

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

Kwok T, Zabler D, Urman S, Rohde M, Hartig R, Wessler S, Misselwitz R, Berger J, Sewald N, Konig W, Backert S (2007) *Helicobacter* exploits integrin for type IV secretion and kinase activation. *Nature* **449:** 862–866

Lai EM, Kado CI (2000) The T-pilus of *Agrobacterium tumefaciens*. *Trends Microbiol* **8:** 361–369

Lawley TD, Klimke WA, Gubbins MJ, Frost LS (2003) F factor conjugation is a true type IV secretion system. *FEMS Microbiol Lett* **224:** 1–15

Levine MM, Ristaino P, Marley G, Smyth C, Knutton S, Boedeker E, Black R, Young C, Clements ML, Cheney C (1984) Coli surface antigens 1 and 3 of colonization factor antigen II-positive enterotoxigenic *Escherichia coli*: morphology, purification, and immune responses in humans. *Infect Immun* **44:** 409–420

Li CM, Brown I, Mansfield J, Stevens C, Boureau T, Romantschuk M, Taira S (2002) The Hrp pilus of *Pseudomonas syringae* elongates from its tip and acts as a conduit for translocation. *EMBO J* **21:** 1909–1915

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

Lu J, Manchak J, Klimke W, Davidson C, Firth N, Skurray RA, Frost LS (2002) Analysis and characterization of the IncFV plasmid pED208 transfer region. *Plasmid* **48:** 24–37

Lundmark K (2005) Protein fibrils in nature can enhance amyloid protein A amyloidosis in mice: cross-seeding as a disease mechanism. *Proc Natl Acad Sci USA* **102:** 6098–6102

Macnab R (2004) Type III flagellar protein export and flagellar assembly. *Biochim Biophys Acta Mol Cell Res* **1694:** 207–217

Maier B, Potter L, So M, Long CD, Seifert HS, Sheetz MP (2002) Single pilus motor forces exceed 100 pN. *Proc Natl Acad Sci USA* **99:** 16012–16017

Marlovits TC, Kubori T, Lara-Tejero M, Thomas D, Unger VM, Galan JE (2006) Assembly of the inner rod determines needle length in the type III secretion injectisome. *Nature* **441:** 637–640

Marlovits TC, Kubori T, Sukhan A, Thomas DR, Galan JE, Unger VM (2004) Structural insights into the assembly of the type III secretion needle complex. *Science* **306:** 1040–1042

Marvin DA, Folkhard W (1986) Structure of F-pili: reassessment of the symmetry. *J Mol Biol* **191:** 299–300

Merz AJ, So M, Sheetz MP (2000) Pilus retraction powers bacterial twitching motility. *Nature* **407:** 98–102

Moore D, Hamilton CM, Maneewannakul K, Mintz Y, Frost LS, Ippen-Ihler K (1993) The *Escherichia coli* K-12 F plasmid gene traX is required for acetylation of F pilin. *J Bacteriol* **175:** 1375–1383

Mu XQ, Bullitt E (2006) Structure and assembly of P-pili: a protruding hinge region used for assembly of a bacterial adhesion filament. *Proc Natl Acad Sci USA* **103:** 9861–9866

Mueller CA, Broz P, Muller SA, Ringler P, Erne-Brand F, Sorg I, Kuhn M, Engel A, Cornelis GR (2005) The V-antigen of *Yersinia* forms a distinct structure at the tip of injectisome needles. *Science* **310:** 674–676

Muller SA, Pozidis C, Stone R, Meesters C, Chami M, Engel A, Economou A, Stahlberg H (2006) Double hexameric ring assembly of the type III protein translocase ATPase HrcN. *Mol Microbiol* **61:** 119–125

Ng TW, Akman L, Osisami M, Thanassi DG (2004) The usher N terminus is the initial targeting site for chaperone–subunit complexes and participates in subsequent pilus biogenesis events. *J Bacteriol* **186:** 5321–5331

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

Nishiyama M, Ishikawa T, Rechsteiner H, Glockshuber R (2008) Reconstitution of pilus assembly reveals a bacterial outer membrane catalyst. *Science* **320:** 376–379

Nuccio S, Baumler A (2007) Evolution of the chaperone/usher assembly pathway: fimbrial classification goes Greek. *Microbiol Mol Biol Rev* **71:** 551–575

Nunn DN, Lory S (1991) Product of the *Pseudomonas aeruginosa* gene pilD is a prepilin leader peptidase. *Proc Natl Acad Sci USA* **88:** 3281–3285

Olsen A, Jonsson A, Normark S (1989) Fibronectin binding mediated by a novel class of surface organelles on *Escherichia coli*. *Nature* **338:** 652–655

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

Remaut H, Rose RJ, Hannan TJ, Hultgren SJ, Radford SE, Ashcroft AE, Waksman G (2006) Donor-strand exchange in chaperone-assisted pilus assembly proceeds through a concerted strand displacement mechanism. *Mol Cell* **22:** 831–842

Remaut H, Tang C, Henderson NS, Pinkner J, Wang T, Hultgren SJ, Thanassi DG, Waksman G, Li H (2008) Fibre formation across the bacterial outer membrane by the chaperone/usher pathway. *Cell* **33:** 640–652

Robinson LS, Ashman EM, Hultgren SJ, Chapman MR (2006) Secretion of curli fibre subunits is mediated by the outer membrane-localized CsgG protein. *Mol Microbiol* **59:** 870–881

Roine E, Wei W, Yuan J, Nurmiaho-Lassila EL, Kalkkinen N, Romantschuk M, He SY (1997) Hrp pilus: an hrp-dependent bacterial surface appendage produced by *Pseudomonas syringae* pv. tomato DC3000. *Proc Natl Acad Sci USA* **94:** 3459–3464

Sagulenko E, Sagulenko V, Chen J, Christie PJ (2001a) Role of *Agrobacterium* VirB11 ATPase in T-pilus assembly and substrate selection. *J Bacteriol* **183:** 5813–5825

Sagulenko V, Sagulenko E, Jakubowski S, Spudich E, Christie PJ (2001b) VirB7 lipoprotein is exocellular and associates with the *Agrobacterium tumefaciens* T pilus. *J Bacteriol* **183:** 3642–3651

Salih O, Remaut H, Waksman G, Orlova EV (2008) Structural analysis of the Saf pilus by electron microscopy and image processing. *J Mol Biol* **379:** 174–187

Satyshur KA, Worzalla GA, Meyer LS, Heiniger EK, Aukema KG, Misic AM, Forest KT (2007) Crystal structures of the pilus retraction motor PilT suggest large domain movements and subunit cooperation drive motility. *Structure* **15:** 363–376

Sauer FG, Fütterer K, Pinkner JS, Dodson KW, Hultgren SJ, Waksman G (1999) Structural basis of chaperone function and pilus biogenesis. *Science* **285:** 1058–1061

Sauer FG, Pinkner JS, Waksman G, Hultgren SJ (2002) Chaperone priming of pilus subunits facilitates a topological transition that drives fiber formation. *Cell* **111:** 543–551

Sauer FG, Remaut H, Hultgren SJ, Waksman G (2004) Fiber assembly by the chaperone–usher pathway. *Biochim Biophys Acta* **1694:** 259–267

Schmidt-Eisenlohr H, Domke N, Angerer C, Wanner G, Zambryski PC, Baron C (1999) Vir proteins stabilize VirB5 and mediate its association with the T pilus of *Agrobacterium tumefaciens*. *J Bacteriol* **181:** 7485–7492

Schroder G, Lanka E (2005) The mating pair formation system of conjugative plasmids-A versatile secretion machinery for transfer of proteins and DNA. *Plasmid* **54:** 1–25

Sekiya K, Ohishi M, Ogino T, Tamano K, Sasakawa C, Abe A (2001) Supermolecular structure of the enteropathogenic *Escherichia coli* type III secretion system and its direct interaction with the EspA-sheath-like structure. *Proc Natl Acad Sci USA* **98:** 11638–11643

So SS, Thanassi DG (2006) Analysis of the requirements for pilus biogenesis at the outer membrane usher and the function of the usher C-terminus. *Mol Microbiol* **60:** 364–375

Strom MS, Lory S (1987) Mapping of export signals of *Pseudomonas aeruginosa* pilin with alkaline phosphatase fusions. *J Bacteriol* **169:** 3181–3188

Tamano K, Aizawa S, Katayama E, Nonaka T, Imajoh-Ohmi S, Kuwae A, Nagai S, Sasakawa C (2000) Supramolecular structure of the *Shigella* type III secretion machinery: the needle part is changeable in length and essential for delivery of effectors. *EMBO J* **19:** 3876–3887

Turner LR, Lara JC, Nunn DN, Lory S (1993) Mutations in the consensus ATP-binding sites of XcpR and PilB eliminate extracellular protein secretion and pilus biogenesis in *Pseudomonas aeruginosa*. *J Bacteriol* **175:** 4962–4969

Verger D, Bullitt E, Hultgren SJ, Waksman G (2007) Crystal structure of the P pilus rod subunit PapA. *PLoS Pathog* **3:** e73

Verger D, Miller E, Remaut H, Waksman G, Hultgren S (2006) Molecular mechanism of P pilus termination in uropathogenic *Escherichia coli. EMBO Rep* **7:** 1228–1232

Vetsch M, Puorger C, Spirig T, Grauschopf U, Weber-Ban EU, Glockshuber R (2004) Pilus chaperones represent a new type of protein-folding catalyst. *Nature* **431:** 329–333

White AP, Collinson SK, Banser PA, Gibson DL (2001) Structure and characterization of AgfB from *Salmonella enteritidis* thin aggregative fimbriae. *J Mol Biol* **311:** 735–749

Woestyn S, Allaoui A, Wattiau P, Cornelis GR (1994) YscN, the putative energizer of the *Yersinia* Yop secretion machinery. *J Bacteriol* **176:** 1561–1569

Wolfgang M, van Putten JP, Hayes SF, Dorward D, Koomey M (2000) Components and dynamics of fiber formation define a ubiquitous biogenesis pathway for bacterial pili. *EMBO J* **19:** 6408–6418

Wright KJ, Seed PC, Hultgren SJ (2007) Development of intracellular bacterial communities of uropathogenic *Escherichia coli* depends on type 1 pili. *Cell Microbiol* **9:** 2230–2241

Yeo HJ, Yuan Q, Beck MR, Baron C, Waksman G (2003) Structural and functional characterization of the VirB5 protein from the type IV secretion system encoded by the conjugative plasmid pKM101. *Proc Natl Acad Sci USA* **100:** 15947–15952

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

Zavialov AV, Berglund J, Pudney AF, Fooks LJ, Ibrahim TM, MacIntyre S, Knight SD (2003) Structure and biogenesis of the capsular F1 antigen from *Yersinia pestis* preserved folding energy drives fiber formation. *Cell* **113:** 587–596

Zogaj X, Nimtz M, Rohde M, Bokranz W, Romling U (2001) The multicellular morphotypes of *Salmonella typhimurium* and *Escherichia coli* produce cellulose as the second component of the extracellular matrix. *Mol Microbiol* **39:** 1452–1463