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Review

# The molecular dissection of the chaperone–usher pathway ☆

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ABSTRACT

Secretion systems are specialized in transport of proteins, DNA or nutrients across the cell envelope of bacteria and enable them to communicate with their environment. The chaperone–usher (CU) pathway is used for assembly and secretion of a large family of long adhesive protein polymers, termed pili, and is widespread among Gram-negative pathogens [1]. Moreover, recent evidence has indicated that CU secretion systems are also involved in sporulation [2,3]. In this review we focus on the structural biology of the paradigmatic type 1 and P pili CU systems encoded by uropathogenic *Escherichia coli* (UPEC), where recent progress has provided unprecedented insights into pilus assembly and secretion mechanism. This article is part of a Special Issue entitled: Protein trafficking and secretion in bacteria. Guest Editors: Anastassios Economou and Ross Dalbey.



## 1. Background

To gain an initial foothold, UPEC strains use a repertoire of adhesive pili including S pili, Dr pili, P pili and type 1 pili to attach themselves to various parts of the urinary tract [4]. Thus, the expression and tissue specificity of these pili determine the tropism of UPEC strains within the urinary tract. CU pili protrude from the bacterial surface as micrometer long fibres. Adhesins on their tips mediate specific binding to the epithelium of the urinary tract: type 1 pilus adhesin FimH binds mannosylated receptors on the bladder epithelium while P pilus adhesin PapG binds to Gal-α(1–4)-Gal-containing glycolipid receptors on renal tissue [5–8]. Consequently, the expression of type 1 and P pili has been linked to bladder (cystitis) and kidney (pyelonephritis) infections, respectively [9–11]. Once comfortably niched in the bladder epithelium, UPEC can lie dormant to cause recurrent infections or replicate rapidly forming biofilm-like, type 1 pili-dependent, intracellular bacterial communities (IBCs). Upon IBC maturation, bacteria disperse and spread to neighboring cells where they re-launch the infection process and form the next-generation IBCs, explaining the highly recurrent nature of cystitis [12].

## 2. Pilus architecture

CU pili are anchored in the outer bacterial membrane by their secretion and assembly machine, the usher (Fig. 1). P pili and type 1 pili exhibit right-handed helical rod-like shapes (3.3 subunits per turn) [13,14] with a short flexible fibrillum on top [5,8]; their rods are made of ~1000 copies of the major pilus rod subunits PapA or FimA, respectively [15,16]. The Pap tip fibrillum consists of one copy of PapK, followed by 5–10 copies of PapE, and one copy each of PapF and the adhesin PapG [5]. The type 1 pilus fibrillum is made of single copies of subunits FimF, FimG and the adhesin FimH [15].

## 3. Adhesins

As opposed to the other CU pilus subunits, which contain only one domain (termed "pilin domain"), adhesins contain, in addition, an N-terminal lectin domain. Adhesins are connected to other pilus subunits via their pilin domain, while the lectin domain mediates binding to host surface structures. Since pilus biogenesis cannot be initiated in their absence, adhesins are always assembled into the pilus first and therefore locate to the distal end of the pilus. Although their lectin domains share a β-barrel jelly-roll fold, their receptor binding sites differ markedly [17–21]. The D-Mannopyranoside binding site of adhesin FimH is a deep negatively charged pocket at the tip of the lectin domain [19] (Fig. 2a). In contrast, the Gal(α1–4)Gal binding site of PapGII (class II of three PapG classes binding different globoseries of glycolipids) is a shallow pocket composed of three β-strands and a loop on the side of the molecule (Fig. 2b) [18].

## 4. The chaperone usher pilus assembly pathway

Pilus subunits are secreted through the inner membrane by the SecYEG translocon (Fig. 1) [22]. CU chaperones bind to subunits as

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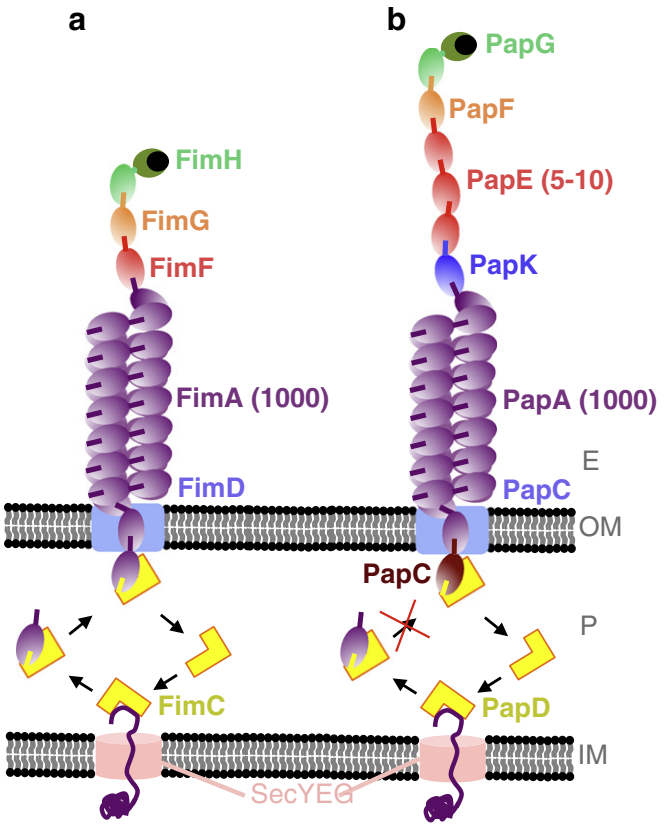


**Fig. 1.** Schematics of type 1 (a) and P pili (b). The ushers FimD and PapC are represented as blue rectangles spanning the outer membrane (OM) and pilus subunits as oval shapes. Chaperones FimC and PapD are drawn as yellow L-shapes. The SecYEG translocon is shown as pink barrel in the inner membrane (IM). Extracellular space (E), periplasm (P).

they exit the translocon and catalyze their folding via a mechanism that is termed 'Donor Strand Complementation'. Then chaperone:subunit complexes are recruited to the usher situated in the outer membrane for assembly into a pilus. The usher catalyzes the ordered assembly of pilus subunits via the 'Donor Strand Exchange' mechanism [23,24] and secretes the nascent pilus into the extracellular space across the outer bacterial membrane.

### 4.1. Donor strand complementation

Pilus subunits exhibit an incomplete Imunoglobulin (Ig)-like fold. An Ig-like fold usually contains 7 β-strands arranged in a β-sandwich. Pilus subunits lack the seventh C-terminal β-strand (strand G) and therefore are made of only six β-strands (A-F) [25,26]. The absence of this β-strand creates a hydrophobic groove and causes subunits to misfold [27,28].

CU chaperones have a conserved boomerang-like shape consisting of two Ig-like domains. They stabilize the incomplete subunit's Ig-like fold by inserting their G1 β-strand (strand G of the chaperone's domain 1) into the hydrophobic groove of the pilus subunit resulting in stable binary chaperone:subunit complexes (Fig. 3a, b). The process of stabilizing the pilus subunit is termed donor strand complementation (DSC) because the CU chaperones complement the incomplete subunit's Ig-like fold by donating the missing β-strand [25,26]. While in a regular Ig fold, the seventh strand G runs antiparallel to strand F, the donor β-strand G1 of the chaperone runs parallel to the subunit's F β-strand. Thus, the chaperone-complemented Ig-fold of the subunit is atypical (Fig. 3c). The G1 donor strand of CU chaperones contains four conserved alternating hydrophobic residues, named P1–P4, which occupy four conserved pockets (termed P1–P4 as well) in the hydrophobic groove of the pilus subunit (Fig. 3a, b). A fifth hydrophobic pocket, P5 in the hydrophobic groove remains accessible for polymerization with the next subunit (see next section) (Fig. 3a, b).

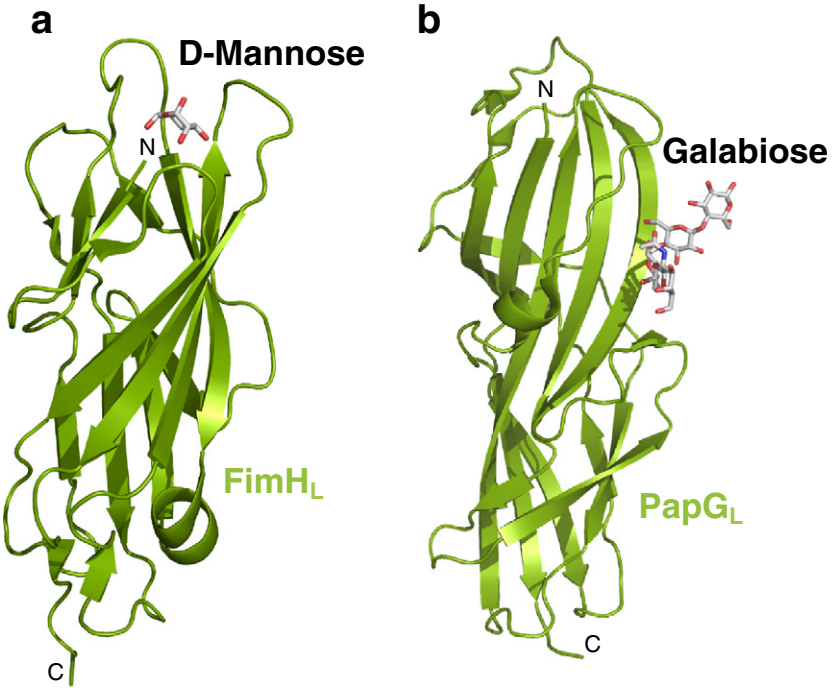


**Fig. 2.** Receptor binding by lectin domains. All proteins are shown as ribbon and bound molecules as stick models. (a) Crystal structure of the FimH lectin domain (FimH$_L$) bound to α-D-Mannose. (b) Crystal structure of the PapG lectin domain (PapG$_L$) in complex with GalNAc β 1–3Gal α 1–4Gal β 1–4Glc (Galabiose).

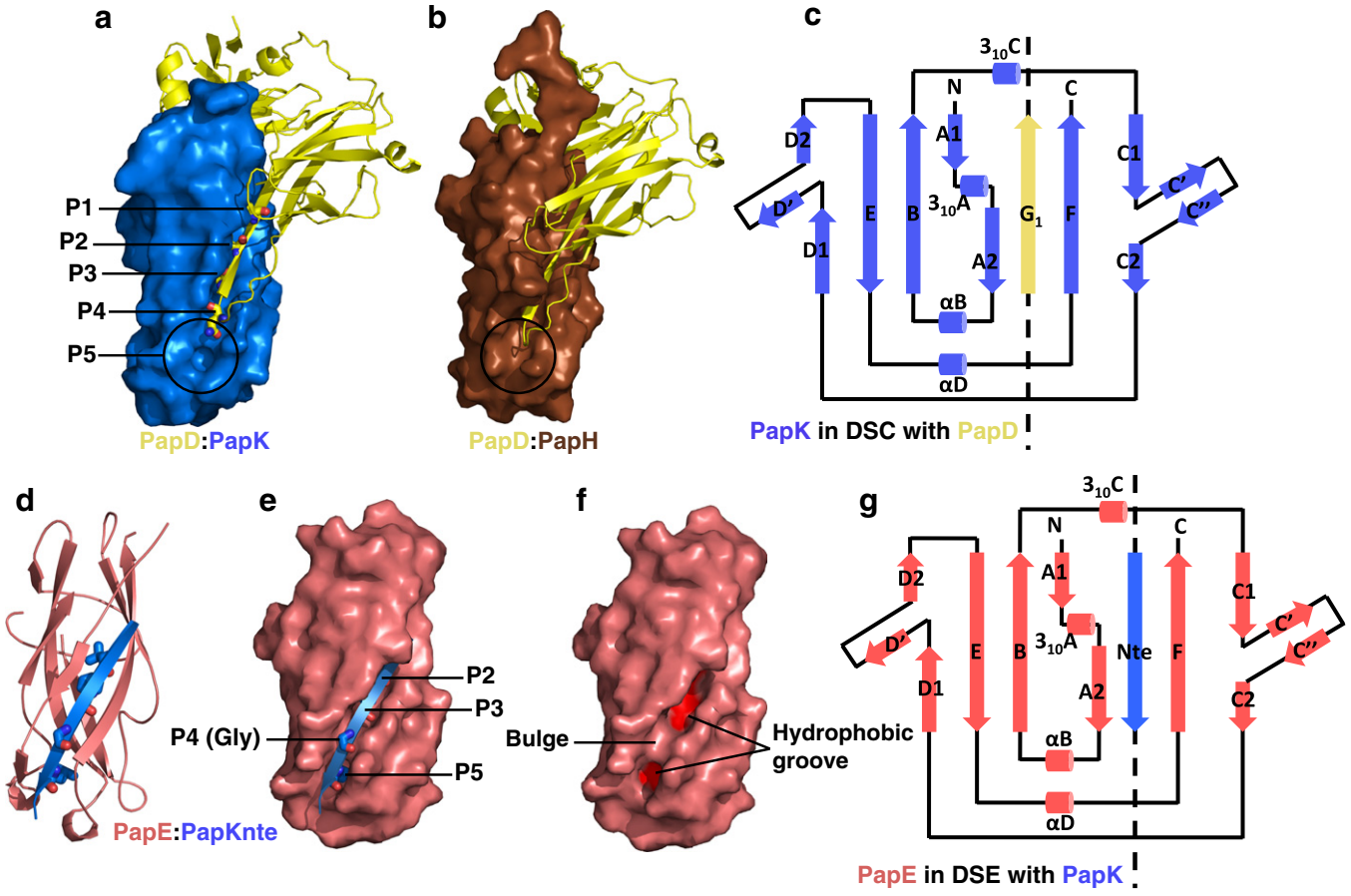


**Fig. 3.** Mechanisms of CU pilus assembly: donor strand complementation (DSC) and donor strand exchange (DSE). (a) Crystal structure of P pilus subunit PapK bound to chaperone PapD (Donor Strand Complementation). PapK (blue) is shown as surface representation and PapD (yellow) as ribbon model. The structurally conserved residues P2–P4 of PapD are presented as sticks. The PapK P5 pocket is indicated in a black open circle. (b) Crystal structure of P pilus termination- and anchor subunit PapH bound to chaperone PapD. PapH (brown) is shown as brown surface model and PapD (yellow) as ribbon model. The black open circle indicates where the P5 pocket would be expected. (c) Topology diagram of P pilus subunit PapK (blue) complemented with the G1 strand of chaperone PapD (yellow). (d, e, f) Crystal structure of P pilus subunit PapE bound to the Nte of PapK (Donor Strand Exchange). PapE is presented as ribbon (d) and surface model (e, f). The Nte of PapK (blue) is shown as ribbon model. Structurally conserved residues P1–P4 of PapD are presented as stick model. Surface representation of the subunit (f) shows the bulge (created by Phe 138) that acts as a control for positioning the incoming Nte. The Nte of PapK was removed for clarity. (g) Topology diagram of P pilus subunit PapE (red) with the Nte of the following P pilus subunit PapK (blue).

### *4.2. Donor strand exchange*

At the outer membrane the usher catalyzes the polymerization of pilus subunits in an ordered fashion (see section 'subunit ordering') [24]. Subunits are incorporated into the growing pilus one at a time, in successive subunit-incorporation cycles, whereby the next subunit in assembly reacts/polymerizes with the subunit incorporated in the previous cycle [23].

For polymerization, pilus subunits use disordered 10–20 residues long N-terminal extensions (Nte), which are not part of their Ig-like fold. The usher catalyzes the displacement of the chaperone's donor strand from the last assembled pilus subunit (also termed "acceptor" subunit) and replaces it with the Nte of the next pilus subunit in assembly (also termed "incoming" subunit). This mechanism of substitution of the chaperone strand for the Nte of the next subunit in assembly is termed 'donor strand exchange' (DSE).

In the resulting pilus polymer, subunits are connected by these Ntes, each inserted into the hydrophobic grooves of their preceding subunits forming very stable non-covalent, hydrophobic interactions that are among the strongest interactions observed in nature ($1.5 \times 10^{-20}$ M) [29–31]. Ntes contain four conserved alternating hydrophobic residues termed "P2–P5 residues" which occupy four of the five conserved pockets mentioned above, namely P2–P5, in the hydrophobic groove of the previously assembled acceptor subunit (Fig. 3d) [25]. DSE was proposed to work as a "zip-in zip-out" mechanism whereby the Nte of the incoming subunit progressively displaces the G1 donor β-strand of the chaperone, proceeding from pocket P5 to P2 [32,33].

The correct positioning of the incoming subunit's Nte in the acceptor subunit's groove is ensured by a bulge formed by an aromatic residue in the P4 pocket (Fig. 3e, f). Only residue P4 of the incoming subunit's Nte, a Glycine, which is conserved in all subunits, can be accommodated on top of this bulge as it has no side chain. After DSE, the Nte of the incoming subunit runs anti-parallel to β-strand F of the acceptor subunit and thus, the acceptor subunit now exhibits a canonical *trans*-complemented Ig-fold (Fig. 3g).

## 5. Mechanism of DSE at the usher

Subunit polymerization can occur spontaneously but, in presence of the usher, DSE rates are significantly elevated [24]. The usher-catalyzed DSE reaction is independent from intracellular energy [34]. The required energy for pilus subunit polymerization is stored in the chaperone complemented subunit fold and released upon DSE [23,31,32]. Thermal denaturation experiments have shown that pilus subunits are significantly more stable when complemented by the Nte of their cognate subunit than by the chaperone's Nte. This difference in stabilities results in a free energy potential that drive pilus subunit polymerization [31].

### 5.1. The usher in its inactive state

The usher is a ~90 kDa protein that consists of five domains: an N-terminal domain (NTD), an outer membrane pore, a plug domain and two C-terminal domains (CTD1 and CTD2).

The structure of the pore domain of the PapC usher, the usher that assembles P pili, has been solved [35]. It consists of a large β-barrel, which is composed of 24 β-strands forming a pore occluded by the plug domain [35–37]. Structural information for the N- and C-terminal domains in the inactive resting usher state is currently missing. In its resting state, the usher is dimeric [35–37].

### 5.2. Usher activation and initiation of pilus biogenesis

The crystal structure of an activated usher, that of FimD, the usher that assembles type 1 pili, bound to the chaperone:adhesin, FimC:FimH, complex was instrumental in revealing some aspects of usher activation ([38]; Fig. 4, state 3). Indeed, in this structure, the plug domain has swung out and instead it is the lectin domain of FimH ('$FimH_L$') which is observed inserted inside the pore. Thus, upon binding of the chaperone:adhesin FimC:FimH complex the plug domain is displaced by the lectin domain from the pore lumen into the periplasm, thereby opening the usher pore (Fig. 4, states 1–3) [38]. The exact mechanism for the plug displacement is not well understood. Electrophysiology experiments have indicated that the plug domain in the PapC apo usher can move sporadically out of the usher pore [39]. In support of these data, computer simulations of the FimD usher pore in the Rosetta force field have shown that the plug can exit the usher pore at a low energetic cost. In contrast, in the FimD:FimC:FimH complex, extrusion of the FimH lectin domain from the usher pore encounters a steep energy barrier suggesting that the lectin domain competes off the plug domain and does not let it back in [40].

Concomitantly with the displacement of the plug domain, the usher pore undergoes a conformational change from a kidney-shape to a more circular shape in order to accommodate the FimH lectin domain and the subsequent subunits (the nascent pilus) passing through the pore (compare Fig. 5a and b) [24,41]. The new circular usher pore shape leads to the disruption of the dimers the usher forms when inactive. *In vitro* experiments and recent crystallographic data strongly suggest that the functional/active form of the usher is indeed monomeric [36,38,40,42].

## 6. Pilus assembly

The usher has two periplasmic binding sites for chaperone:subunit complexes. One is formed by the usher N-terminal domain (NTD) [43–45] and the other one by its two C-terminal domains (CTD1 and CTD2) [38]. The chaperone:adhesin complex FimC:FimH in the FimD:FimC:FimH crystal structure is observed bound to the CTDs, with the NTD free to bind the next chaperone:subunit complex in assembly (Fig. 4, state 3). As shown by modeling, binding of a chaperone:subunit complex at the NTD positions the Nte of this second subunit in a perfect location relative to the first to trigger DSE (Fig. 4, states 4, 5) indicating that the usher catalyzes DSE just by bringing two subunits in close proximity [38]. In the Pap system, biolayer interferometry and non-covalent electrospray ionization mass spectrometry data have indicated that the plug domain, which in the FimD:FimC:FimH complex, is observed interacting with the usher NTD, is also part of the chaperone:subunit recruitment site formed by the NTD [46,47].

Based on structural and biochemical data, a model for the subunit-incorporation cycle catalyzed by the FimD usher was proposed. In this model, the starting state is that represented by the structure of the FimD:FimC:FimH complex where the previously assembled chaperone:subunit (FimC:FimH) complex is bound to the CTDs (Fig. 4, state 3). The next chaperone:subunit complex FimC:FimG is then recruited by the usher NTD (Fig. 4, state 4); this positions the Nte of FimG next to the groove of FimH bound to the CTDs (Fig. 4, state 4). At this stage, DSE can occur leading to dissociation of the chaperone that was bound to FimH (Fig. 4, state 5). This frees the CTDs. At this stage, FimG now reacted to FimH transfers to the CTDs while FimH inserts inside the pore lumen (Fig. 4, state 6). This ends the cycle and another one can start with the recruitment of the next chaperone:subunit complex in assembly (FimC:FimF) to the usher NTD [38]. We believe, the specific steps described here can be generalized for other CU systems.

Additional evidence for the proposed mechanism was obtained when the structure of the FimD:FimC:FimF:FimG:FimH complex was determined showing a snapshot of the usher as it secretes the entire type 1 pilus tip fibrillum through the usher pore (Fig. 4, state 9) [40]. In this complex the last assembled chaperone subunit complex FimC:FimF is found at the CTDs, confirming that, after DSE, all subunits are transferred to the usher CTDs.

How this NTD-to-CTDs transfer of chaperone:subunit complexes operates is not fully understood. One of the pre-conditions for transfer is the dissociation of the incoming chaperone:subunit complex from the usher NTD upon DSE. Without it, the complex would remain "stuck" at the usher NTD. Interestingly, Volkan et al. have shown that CTD2 alone is capable of catalytically dissociating NTD-bound chaperone:subunit complexes from the NTD [47]. Thus CTD2 might be one of the wheels in the mechanism of transfer. However, once dissociated from the NTD, other players must be involved to guide the nascent pilus to the CTDs and translocate the previously-assembled subunit through the pore.

One of these players was recently identified by Geibel et al. [40]. The handover step requires an upward motion of 53 Å and a simultaneous counter clockwise rotation of the nascent pilus of about 110° (Fig. 6). Simulation of subunit transport (applied to FimG in the usher pore of the FimD:FimC:FimF:FimG:FimH complex crystal structure) along the usher pore using the Rosetta force field revealed a helical low energy path similar to a screw thread along which subunits can enter and exit the usher pore [40]. Strikingly, this energy path imposes a rotational and translational motion on the transported subunit, which matches exactly what is required for the translocating nascent pilus to execute the NTD to CTDs transfer mentioned above. Hence, the low energy path energetically facilitates not only the transport through the pore but also the handover motion.

However, no low energy path was observed for the lectin domain of FimH, which might not be surprising as this domain has a very different structure compared to that of all other subunits. Moreover, in the same study, it was shown that the lectin domain of FimH interacts strongly with the pore lumen, likely in order to compete off the plug domain during usher activation. Thus, if there were a low energy track for the FimH lectin domain, it would be subsumed within the noise of the high affinity binding energy that the domain displays for the usher pore lumen and thus would not be observed.

Thus, subunits are guided through the pore lumen by the low energy path in such a way that they must translate and rotate in a motion that facilitates transfer of the nascent pilus from the usher NTD to the usher CTDs. However, what is still unclear is what pulls or pushes the subunits through the pore. The low energy path does not provide an energy gradient by which subunit transport could be driven. Our own favoured hypothesis is that subunits are pulled through the pore, and not pushed. How would a pulling mechanism work? Comparing the structures of the FimD:FimC:FimH and FimD:FimC:FimF:FimG:FimH complexes, we were able to observe FimH before and after translocation and noted a large change in the angle that the two domains of FimH, $FimH_L$ and $FimH_P$ make with each other (Fig. 7a): when inside the pore, the two domains are aligned (angle between the two domains of 180°) while, once exited from the pore, the angle between the two domains decreases by 37.5°. We hypothesize that this new conformation would prevent FimH and the nascent pilus from back-sliding.

Thus, subunits might be free to move in and out within the usher pore, but inward motions would be buffered by the usher periplasmic

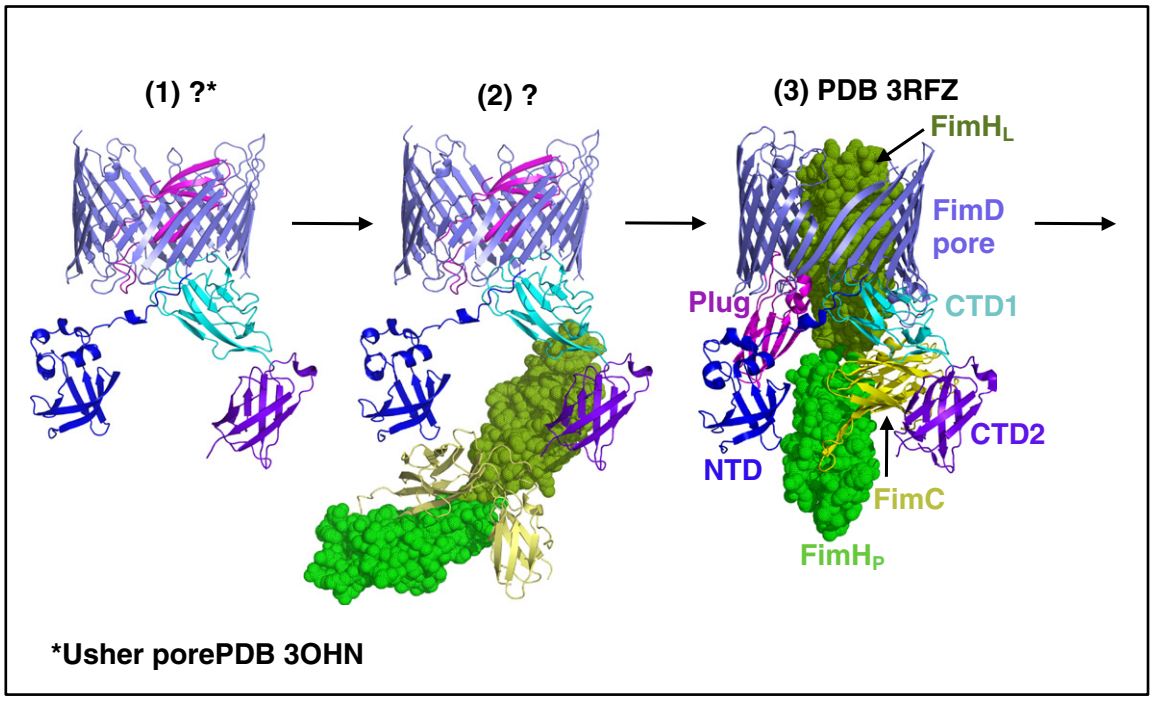


**Fig. 4.** Model of the subunit incorporation cycle catalyzed by the FimD usher. The usher FimD and the chaperone FimC are shown as ribbon models using the following color code: FimD NTD (blue), β-barrel (slate), plug domain (magenta), CTD1 (cyan) and CTD2 (purple); chaperone (yellow). Type 1 pilus subunits are shown in sphere representation with the following colors: FimH lectin domain ((FimH$_L$, dark green) and pilin domain (FimH$_P$, green), FimG (orange) and FimF (red). Pilus biogenesis intermediate states for which no structures are available are indicated by a question mark. The first structure on the top left corner is also a hypothetical model as the full-length FimD structure has not been crystallized (state 1). For this state, only the pore domain has been crystallized and its structure determined. The corresponding PDB entry code is indicated by a star. Recruitment of FimC:FimH (state 2) results in the pilus biogenesis initiation complex FimD:FimC:FimH (state 3). Elongation of the nascent pilus starts upon recruitment of FimC:FimG to the FimD NTD (state 4). The unstructured Nte of FimG is indicated as dashed line. Binding to the usher NTD brings FimG (and its Nte) in close proximity to FimH triggering donor-strand exchange (DSE) and dissociation of the chaperone FimC from the pilin domain of FimH (state 5). The nascent pilus is handed over from the NTD to the CTDs and is translocated upwards through the pore (state 6). The next cycle of subunit incorporation can now start again: indeed, the usher NTD is now available for binding of the next chaperone:subunit complex in assembly, FimC:FimF. After DSE and transfer to the CTDs, a stable FimD:FimC:FimF:FimG:FimH complex can be obtained and crystallized. Its structure is shown on the lower right corner of the figure (state 9). This structure is now poised to enter another cycle of subunit incorporation, this time with FimC:FimA.

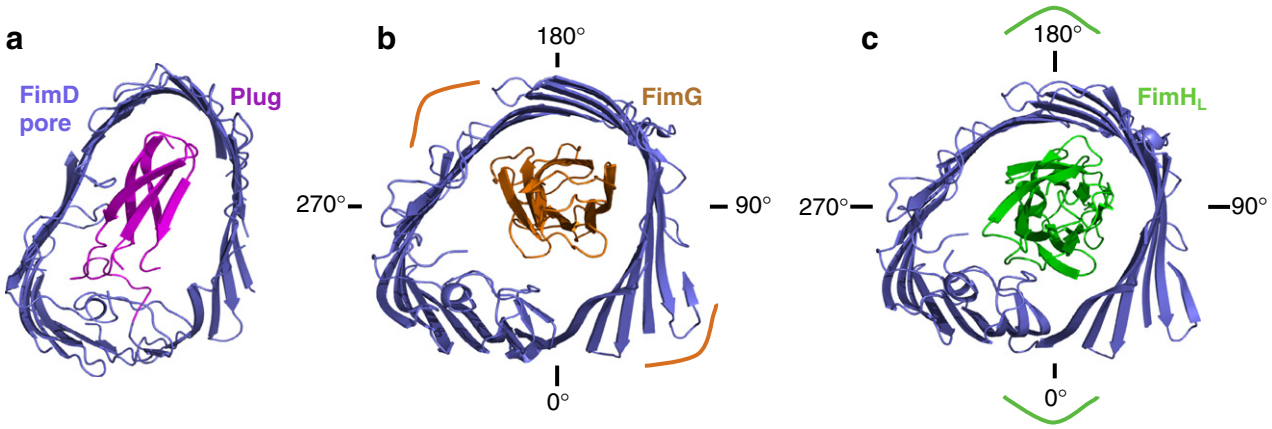


**Fig. 5.** Structural changes of the FimD usher pore upon activation and binding sites of Fim subunits within the usher pore. All proteins are shown in ribbon style. FimD usher pore, FimH lectin domain (FimH$_L$) and linker subunit FimG are colored slate, green and orange, respectively. (a) Crystal structure of the FimD usher pore in its resting state occluded by the plug domain. (b) The FimD usher pore accommodating pilus tip subunit FimG found in complex FimD:FimC:FimF:FimG:FimH and (c) the lectin domain of FimH of the FimD:FimC:FimH complex. In panels b and c, the reference points for angles are indicated. The FimH lectin domain's strongest interactions with the pore lumen residues are within 60° wide segments (indicated by green and orange lines, respectively) which center around ~0° and 180° for FimH$_L$ and around ~60° and 240° for FimG.

domains, whereas, once out of the pore, the nascent pilus would be prevented from back sliding by the conformational change affecting FimH. Similarly, it has been shown that when FimA, the main pilus rod subunit, polymerizes, it forms a wound, right-handed, super-helical structure of 3.3 subunits per turn as FimA subunits emerge from the pore: formation of this super-helical structure would also prevent back-sliding of the growing pilus and therefore drive the translocation of polymerizing subunits outwards.

Analysis of the interface between usher pore and inserted subunits has shown that usher pores have evolved diametrically opposed binding sites for individual subunits. In the FimD:FimC:FimH complex the two opposing binding sites for the lectin domain are at 0° and 180° and in the FimD:FimC:FimF:FimG:FimH complex at 60° and 240° for subunit FimG (Fig. 5b, c). This results in the placement of the translocating substrate at the very center of the pore, which might also further facilitate its translocation.

## 7. Structural changes of FimH after secretion

As mentioned above, prior transport, the chaperone FimC and the insertion of the lectin domain of FimH inside the pore of FimD imposes constraints on the orientation of the two domains of FimH, whereby the

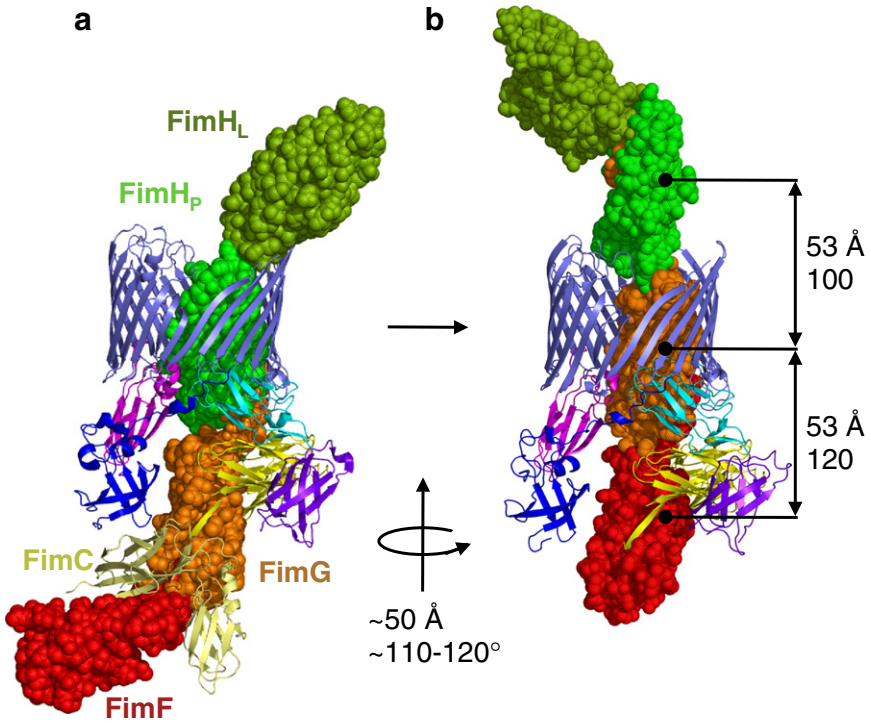


**Fig. 6.** Model for the subunit transfer from the usher NTD to CTDs. All subunits are shown in sphere representation. The FimD usher and the chaperone FimC are presented in ribbon style. The same color-coding as in Fig. 4 was used. (a) Recruitment of chaperone subunit complex FimC:FimF (b) Crystal structure of the FimD:FimC:FimF:FimG:FimH complex obtained after FimC:FimF has undergone DSE with FimG and has transferred to the CTDs. This requires a counterclockwise rotation of ~110°–120° and an upward motion of ~50 Å.

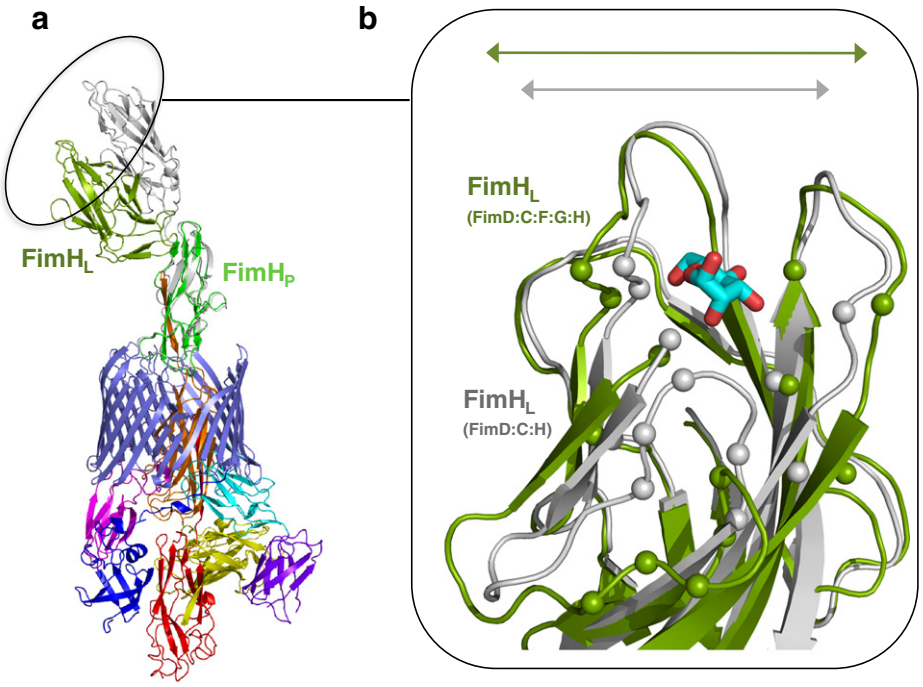


**Fig. 7.** Conformational change of FimH after secretion. All protein are presented in ribbon style. Color coding is as in Fig. 4. (a) Crystal structure of the complex FimD:FimC:FimF:FimG:FimH showing full length FimH entirely emerged from the usher pore in its auto-inhibited, bent conformation (after DSE). Superimposition of FimH of the complex FimD:FimC:FimH in its elongated conformation (grey) reveals the large conformational change FimH undergoes after secretion. (b) Superimposition of FimH lectin domain (dark green) of complex FimD:FimC:FimF:FimG:FimH with FimH lectin domain (grey) of complex FimD:FimC:FimH shows the allosteric induced expansion of the sugar-binding site upon secretion (indicated by the top grey and green arrows). Mannose binding residues are shown as spheres and D-Mannose as stick model (cyan).

long axes of the two domains are aligned [38]. However, after secretion, the two domains of FimH come closer in and adopt the bent shape observed in the FimD:FimC:FimF:FimG:FimH complex (Fig. 7a) [40]. This conformational change results in a more open configuration of the mannose-binding site at the tip of the lectin domain of FimH. Mannose-binding residues within the site move further apart, resulting in a low affinity mannose-binding site (Fig. 7b) [48]. Interestingly, this auto-inhibited conformation is reversed upon applying tensile forces (by using for example an atomic force microscope) that stretch the two domains, reversing the structural changes within the binding site and allowing the mannose binding pocket to tighten up around the mannose. This allosteric catch bond mechanism could prove useful in the presence of urine flow, preventing UPEC from being flushed off, while in the absence of urine flow, UPEC can dissociate from mannosylated receptors and spread to other areas of the urinary tract.

## 8. Subunit ordering

Pilus subunits assemble in a defined order [5,15,49,50]. In all CU pili the first subunit to be assembled is the adhesin as it is required for priming/activating the usher for pilus biogenesis (see above). Chaperone:adhesin complexes of the type 1 and P pili, FimC:FimH and PapD:PapG, respectively, have the highest affinities for the NTD compared to chaperone:subunit complexes (Table 1). Both the lectin and pilin domains of PapGII have been shown to bind the usher NTD, which might explain the higher affinity of the adhesin to the usher NTD compared to other chaperone:subunit complexes, the adhesin contributing two domains to binding (its lectin and pilin domains) while the others complexes contribute only one domain (their pilin domain) [41,46,47,51].

Once the chaperone adhesin complex is bound to the usher, the assembly order of pilus subunits is determined by their preference to polymerize with their "cognate" pilus subunit, where "cognate" refers here to adjacent subunits in the naturally ordered pilus. In fact, DSE rates occur significantly faster between adjacent pilus subunits. This is not only due to the steric fit between the groove of one subunit and the Nte of another, but also more importantly the fit between the P5 residue of the Nte and the P5 pocket of the acceptor groove. DSE rates also correlate with accessibility of the P5 pocket of pilus subunits. The more flexible and hence accessible the region around the P5 pocket is, the faster the DSE rate is [52–54]; some DSE rates are listed in Table 2 [24,36,55].

Upon completion of the pilus tip (FimF:FimG:FimH), transition to the pilus rod assembly is very slow and is the rate limiting step of pilus biogenesis. The pilus assembly is paused here as the DSE rate between FimF, the last assembled tip subunit and FimA the rod subunit is significantly slower compared to any other subunit pair (~33 min to assemble the first rod subunit FimA) while the entire pilus rod consisting of about ~1000 subunits is assembled in not much more than 1 min (960 min$^{-1}$). Pausing pilus biogenesis after assembly of the tip could give the bacterium enough time to produce a sufficient number of rod subunits to satisfy the expected high demand for rod subunits and to ensure pilus completion.

**Table 1**
Affinities of chaperone:subunit complexes to isolated Fim/Pap usher NTDs. In the Pap system affinities were only detectable for the chaperone:adhesin PapD:PapGII complex.

| | FimC:H/PapD:GII | FimC:G | FimC:F | FimC:A |
|---|---|---|---|---|
| Affinity to NTD | 0.9 μM/3.2 nM | 27 μM | 6.6 μM | 29 μM |

**Table 2**
in vitro DSE rates between subunit pairs in the type 1 pilus system.

| | FimG-H | FimF-G | FimA-F | FimA-A |
|---|---|---|---|---|
| DSE rate (min$^{-1}$) | 171 | 3 | 0.03 | 960 |

## 9. Termination of pilus biogenesis

In the P pilus system pilus biogenesis is terminated by incorporation of the termination subunit PapH [56]. The crystal structure of the chaperone-subunit complex PapD:PapH has shown that PapH has no P5 pocket (Fig. 3b) and therefore is unable to undergo DSE [57]. Moreover, *in vitro* experiments indicated that PapH has no affinity for the NTD and CTD2 but the highest affinity of all chaperone subunit complexes for the plug domain indicating the completed pilus is anchored at the plug domain after incorporation of PapH. In the type 1 pilus system no termination subunit has been identified and as a result, the mechanism of the termination process is still unknown.

## 10. Outlook

Pili are a key target for antibiotics, because they are indispensable tools for bacteria to establish infections. Over the past decade the chaperone–usher assembly pathway has been elucidated in unprecedented mechanistic detail and provided targets for the development of a new generation of antibiotics to fight urinary tract infection. These tailor-made antibiotics target the pilus itself or its assembly and are currently being investigated. 'Mannosides' mimicking D-Mannose have been developed to prevent FimH adherence by competing off their natural mannosylated uroplakin receptor [58–60]; a similar competitive binding approach has been applied using the adhesin PapGII structure [61–63]. The structures of chaperone:subunit complex bound to the isolated usher NTD has led to the development of pilicides (bicyclic 2-pyridones) interrupting the recruitment of chaperone subunit complexes to the usher FimD [64]. 'Coilicides' interfere with the pilus rod affecting its uncoiling and recoiling ability [65]. In the Fim system, the recent crystal structures of pilus biogenesis intermediates have revealed new potential drug targets with the prospect of blocking individual steps during pilus biogenesis [38,40]. Thus, a new generation of antibiotics may emerge that disarm virulence factors and render bacterial pathogens harmless.

Although the field of pilus biogenesis by the chaperone–usher pathway has made considerable progress in the last decade, there are still gaps in our knowledge of the system. We still do not know the conformation of the full-length apo usher (Fig. 4 state 1). The determination of the positions of the NTD and CTDs before FimH engagement will provide further details on the usher activation mechanism, possibly explaining how the lectin domain of FimH expels the plug domain from the pore lumen. The structure of a recruitment complex (Fig. 4 state 4) where a chaperone-subunit complex is bound to the NTD will show how subunits interact with each other before they undergo DSE and provide more mechanistic insights onto the proposed 'Zip-in Zip-out' mechanism. Finally, we still do not understand what drives translocation of pilus subunits through the pore. These important issues will no doubt drive progress in the field for the years to come.

## Conflict of interest statement

The authors declare no conflict of interest.

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