

# Fiber Formation across the Bacterial Outer Membrane by the Chaperone / Usher Pathway

*The following commentary was written by Dr. Clare Sansom and Dr. Han Remaut. The original article was published in the May 16, 2008 issue of Cell [1]: Remaut H, Tang C, Henderson NS, Pinkner JS, Wang T, Hultgren SJ, Thanassi DG, Waksman G & Li H. Cell, 2008, 133, 640-52.*

Pathogenic bacteria that have both an outer and an inner membrane - i.e. that are Gram negative - often recognise and attach to target cells within their hosts through hair-like appendages, or pili, on their surfaces. Gabriel Waksman, head of the Institute of Structural Molecular Biology and of the Departments of Crystallography at Birkbeck and of Structural and Molecular Biology at University College London, has spent many productive years studying the structures and elucidating the mechanisms of action of these pili. Now, in a complex study combining both X-ray crystallography and electron microscopy, Han Remaut in Waksman's group, with collaborators in Stony Brook University and in Washington University - St Louis, has solved the structure of the pilus assembly site on the bacterial outer membrane [1]. This has shed new light on the process of pilus formation.

Some of the best understood pilus systems are found in variants of *E. coli* that infect the human urinary tract. The *E. coli* P pilus recognises and binds to kidney cells, causing pyelonephritis [2]; the type 1 pilus binds to cells in the bladder, causing urinary infections. Both types of pilus have similar structures; they are filaments composed of

many protein subunits, with a narrow distal tip on the end of a more rigid helical rod. The proteins making up each pilus type are named consistently; all P pilus subunits are Pap proteins, whereas all type 1 pilus subunits are Fim proteins. The subunits forming each pilus assemble at an outer membrane-bound protein termed the usher [3].

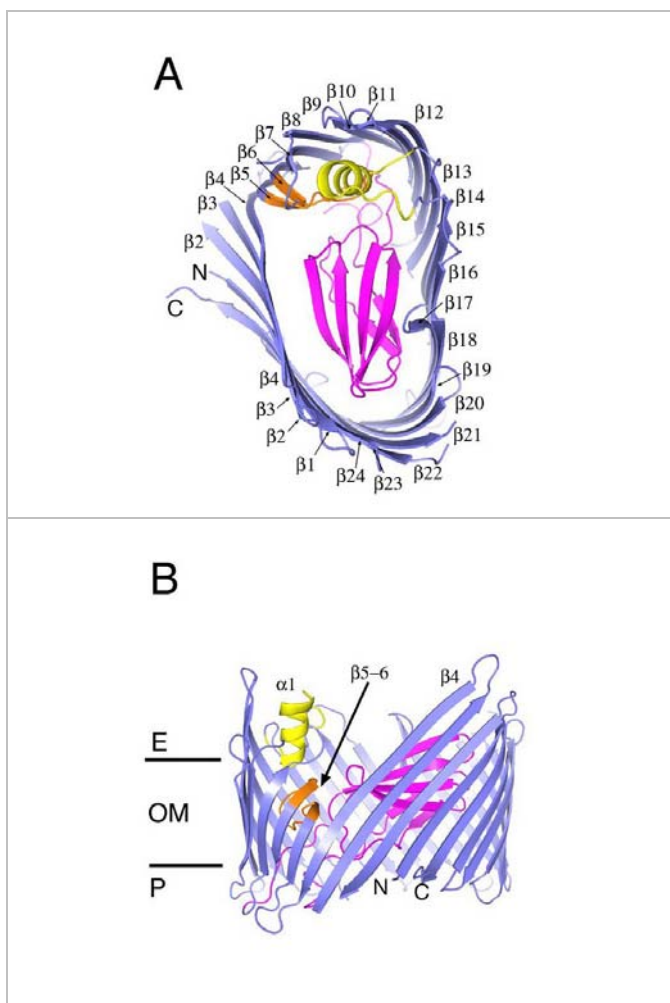
The individual subunits that polymerise to form the filament structures are unstable when isolated. They all share the same immunoglobulin-like fold lacking a single beta strand. After synthesis, they cross the bacterial inner membrane into the periplasmic space between the two membranes, where they bind to a chaperone protein that "donates" a beta strand to the subunit fold to stabilise it [4]. The chaperone-subunit complex is then transported to the usher site, where the subunit is released from its chaperone and joins the growing pilus in a process in which the N-terminal strand of an incoming subunit binds in the gap left in the last assembled subunit by the departing chaperone [5, 6]. This process has been termed donor-strand exchange.

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Although much is known about chaperone and subunit structures and the mechanism of donor-strand exchange, the structure or mechanism of the assembly protein, the usher, has until recently been very poorly understood. Han Remaut and his co-workers have now solved the structure of the P pilus usher using X-ray crystallography.

Outer membrane ushers consist of four domains, with the central part of the protein bound within the outer membrane and N and C terminal domains located within the periplasm. The structure reported here [1] consists of that central region only. This comprises a kidney-shaped, 24-strand beta barrel that is embedded in the membrane, with a separate small domain with a beta-sandwich fold inserted between two of those strands. This is positioned inside the transmembrane beta barrel, blocking its pore, and has hence been termed the plug domain (Figure 1). The protein crystallises as a dimer, which is also its biologically functional form [7].

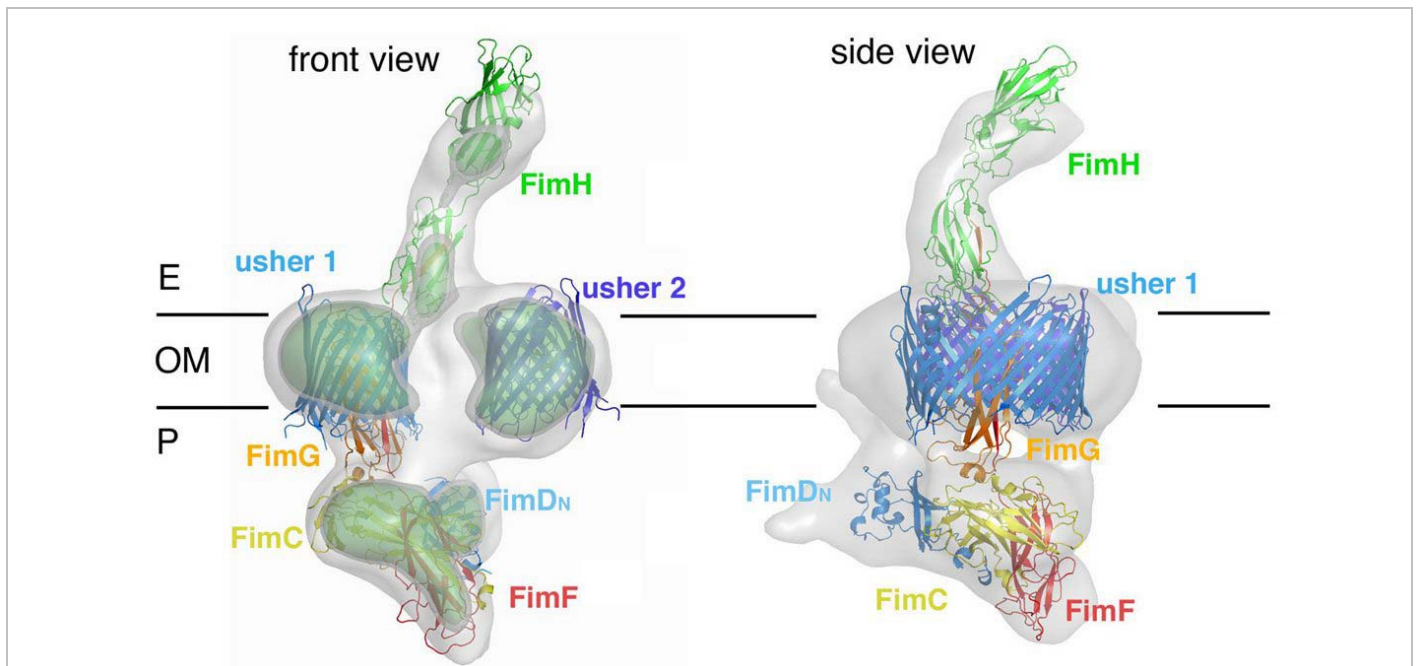


**Figure 1: Crystal structure of PapC<sub>130-640</sub>.** Ribbon representation of the PapC translocation channel viewed from the extracellular side (A) and inside the membrane plane (B). The  $\beta$ -barrel, plug domain,  $\beta$ 5-6 hairpin and helix  $\alpha$ 1 are colored blue, magenta, orange and yellow respectively.  $\beta$ -strands are labeled  $\beta$ 1 through  $\beta$ 24, the labels N and C indicate the N- and C-termini of the translocation channel.

In order to further understand the mechanism of pilus formation, colleagues at Stony Brook isolated the tips of the *E. coli* type 1 pilus “frozen” during secretion through its usher, FimD, for structural studies using cryo-electron microscopy. The resulting electron density map to 23 Angstroms resolution allowed the identification of individual proteins within that structure and the “docking” of previously determined atomic-resolution structures of these proteins into the electron density. The relative positions and orientations of the pilus subunits are appropriate for those subunits to be in donor-strand exchange with each other. Remarkably, although the usher was clearly shown to be a dimer, the density associated with the growing pilus was associated with only one of the monomers, indicating that a single one of the twinned pores is involved in the translocation of the nascent fibre (Figure 2).

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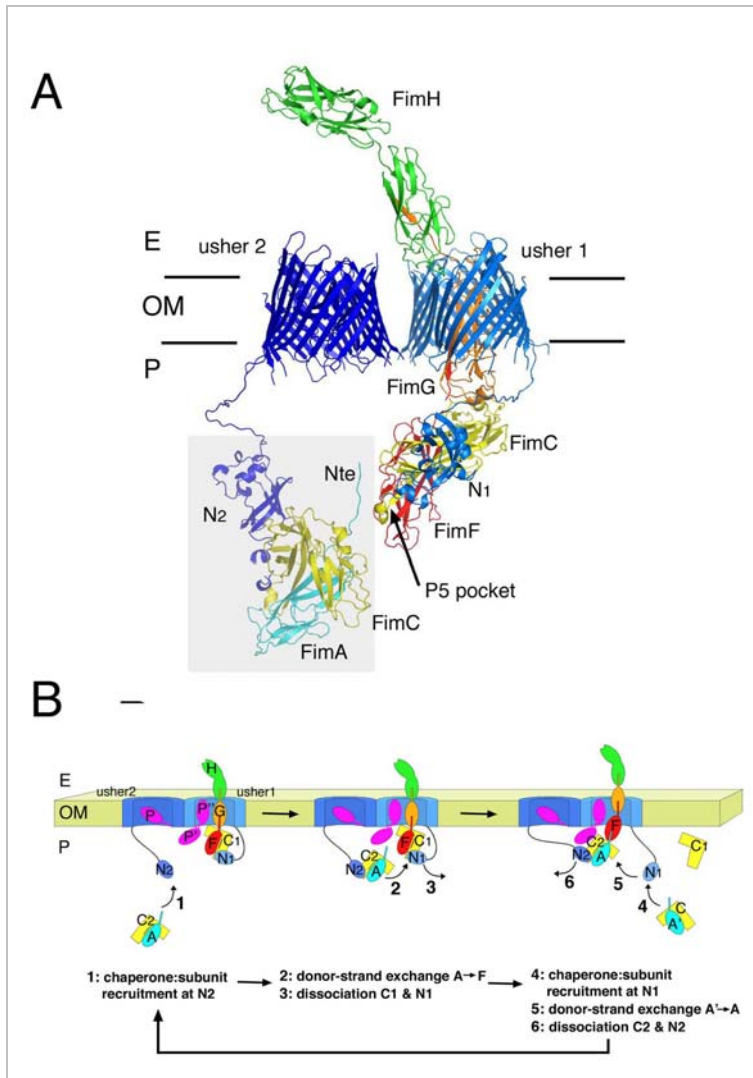
**Figure 2: 3D cryo EM reconstruction of the FimD2:C:F:G:H type 1 pilus tip assembly intermediate.** Front and side views of the 3D reconstruction of the FimD:tip complex rendered at  $2\sigma$  (white surface), and additionally at  $4.5\sigma$  (grey) and  $5\sigma$  (green) in the left view in order to show the EM density's center of mass. Available structures or closely-related models were docked into the EM density as follows: FimH adhesin (green), FimD translocation pore 1 and 2 (blue and dark blue), FimDN:C:F (blue:yellow:red) and FimG (orange). The outer membrane and extracellular and periplasmic space are labeled OM, E and P, respectively.

Based on this new structural data, Remaut and his colleagues have proposed a unifying mechanism for pilus formation that combines what is already known about the molecular biology of the process (Figure 3). In this, the free periplasmic N-terminal domain of one of the twinned usher pores binds the next incoming chaperone-subunit complex. This newly recruited subunit then binds to the previous subunit in the growing pilus via donor-strand exchange, releasing the chaperone and the other usher's N-terminus, which is now in its turn free to recruit the next subunit. Thus, although the fibril grows and is released through only one of the two usher pores, the N-terminal domains of both pores are involved in subunit recruitment via the alternative binding of chaperone-subunit complexes.

Mutant bacteria lacking pili are severely impaired in their ability to cause disease. It is therefore believed that compounds that can block pilus assembly would in effect disarm pathogenic bacteria and be useful as anti-bacterial drugs. Importantly, as these would neutralise, rather than kill, the pathogens, resistance to such compounds would be less likely than resistance to bactericidal drugs to develop. In bringing us closer to understanding the mechanism of pilus formation, this elegant study may have brought us nearer the development of novel and potentially useful antibacterial therapeutics.

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**Figure 3: Model of pilus assembly at the OM usher.** (A) Model of the FimD<sub>2</sub>:C:F:G:H complex as derived by cryoEM including the tentative position (shaded in light grey) of an incoming FimC:A complex bound to usher 2's N-terminal domain (FimD<sub>N2</sub>:C:A, coloured darkblue:yellow:cyan and labeled N2, FimC and FimA, respectively). N1 indicates the N-terminal domain of usher 1. Colors as in Figure 2 (B) Schematic diagram of pilus assembly. At left, the FimD<sub>2</sub>:C:F:G:H complex recruits an incoming FimC:A complex (yellow:cyan) through binding to the N-terminal domain of usher 2 (N2; step 1). The complex is brought within donor-strand exchange of FimF (red), 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 (labeled C:A'; right panel, step 4), 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 fiber (steps 1 through 6).

### References

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