

Review

Fiber assembly by the chaperone–usher pathway

Frederic G. Sauer^a, Han Remaut^b, Scott J. Hultgren^{c,*}, Gabriel Waksman^{b,d,*}

^aSection of Microbial Pathogenesis, Yale University School of Medicine, Boyer Center for Molecular Medicine, 295 Congress Ave., New Haven, CT 06536, USA

^bInstitute of Structural Molecular Biology, School of Crystallography, Birkbeck College, Malet Street, London WC1E 7HX, UK

^cDepartment of Molecular Microbiology, Washington University School of Medicine, Box 8230, 660 S. Euclid, St. Louis, MO 63110, USA

^dDepartment of Biochemistry and Molecular Biology, University College London, Gower Street, London WC1E 6BT, UK

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Abstract

Bacterial pathogens utilize the chaperone–usher pathway to assemble extracellular multi-subunit fibers essential for virulence. The periplasmic chaperone facilitates the initial folding of fiber subunits but then traps them in activated folding transition states. Chaperone dissociation releases the folding energy that drives subunit incorporation into the fiber, which grows through a pore formed by the outer-membrane usher.

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1. Introduction

Gram-negative bacteria, including pathogens such as *Escherichia coli*, *Salmonella enterica* serovars, *Yersinia* species, *Haemophilus influenzae*, *Pseudomonas aeruginosa*, *Bordetella pertussis*, *Klebsiella pneumoniae*, and *Proteus mirabilis*, employ a conserved protein secretion system termed the chaperone–usher pathway to assemble a diverse array of multi-subunit fibers on their surfaces [1–3]. These fibers—termed pili, fimbriae, or fibrillae—range in morphology from relatively thick pilus rods that radiate outward from the bacteria to more flexible pili to very thin fibrillae. Fibers assembled by the chaperone–usher pathway play various and essential roles in bacterial pathogenesis: they mediate microbial attachment to host tissues, they facilitate the evasion of host defenses, and they promote biofilm formation, a contributing factor both to the establishment of infection and to bacterial resistance to antibiotic treatment.

Fibrous organelles assembled by the chaperone–usher pathway are typically encoded in individual gene clusters. Each gene cluster encodes one or more subunit proteins, or pilins, that make up the fiber. Certain pilins interact specifically with receptor molecules and are termed adhesins. Each gene cluster also encodes a periplasmic chaperone and an outer-membrane usher, which together direct the assembly of the fiber. Two well-studied pilus fibers expressed by uropathogenic *E. coli*—P pili and type 1 pili—exemplify the assembly mechanisms of the chaperone–usher pathway. P pili are encoded by the *pap* (pilus associated with pyelonephritis) gene cluster (*papA–K*; Fig. 1A) [4]. P pili bind specifically to the globoseries of glycolipids present in the human kidney and have been shown to be required for the establishment of pyelonephritis, or kidney infection [5,6]. The P pilus consists of a relatively rigid rod, up to 5–7 μm in length, with a thinner, more flexible tip fibrillum, $\sim 42 \pm 16$ nm in length, at its distal end [7] (Fig. 1B). The rod contains PapA subunits wound in a tight, one-start, right-handed helix with a diameter of 6.8 nm, a pitch of 2.5 nm, and 3.3 subunits per turn [8,9]. The interior of the rod contains a channel, roughly 2.5×1.5 nm and with its center displaced ~ 0.5 nm from the helical axis, that winds through the center of pilus. This central cavity is connected to the exterior milieu by a set of radial channels. The tip

* Corresponding authors. Scott J. Hultgren is to be contacted at Department of Molecular Microbiology, Washington University School of Medicine, Box 8230, 660 S. Euclid, St. Louis, MO 63110, USA. Gabriel Waksman, Institute of Structural Molecular Biology, School of Crystallography, Birkbeck College, Malet Street, London WC1E 7HX, UK.

E-mail address: g.waksman@mail.cryst.bbk.ac.uk (G. Waksman).

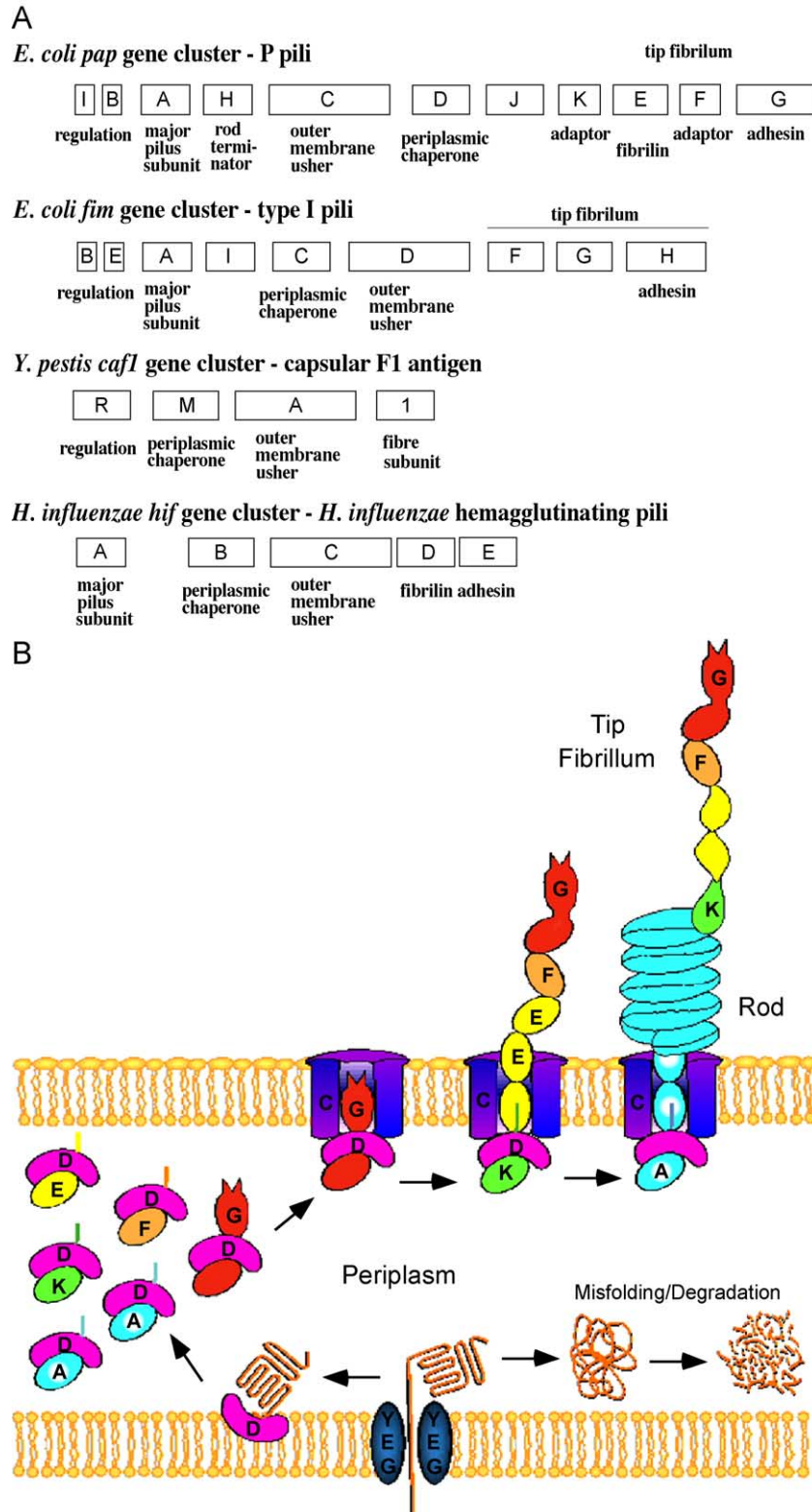


Fig. 1. Organization of the *pap*, *fim*, *cafI* and *hif* chaperone–usher gene clusters and schematic diagram of fiber assembly by the chaperone–usher pathway. (A) Organization of the *pap*, *fim*, *cafI*, and *hif* chaperone–usher gene clusters. (B) Fiber assembly by the chaperone–usher pathway. A schematic of P pilus assembly is shown. Single letters indicate the corresponding Pap protein. Fiber subunits enter the bacterial periplasm via the Sec system (YEG). In absence of the chaperone, subunits misfold, aggregate, and are proteolytically degraded. The chaperone (PapD) forms a soluble complex with each subunit (including the PapG adhesin, PapF, PapE, PapK, and PapA). The chaperone facilitates the folding of the subunit, stabilizes it, and caps its interactive surfaces while priming it for polymerization. Chaperone–subunit complexes are targeted to the pore-forming outer-membrane usher (PapC), where chaperone dissociation permits subunit incorporation into the growing fiber. The pilus adopts its quaternary structure outside the cell.

fibrillum of the P pilus contains PapE subunits arranged in an open helical conformation [7]. The PapG adhesin, which binds to the globoseries of glycolipids, is at the distal end of the tip fibrillum [5,7]. The PapF and PapK subunits are thought to link the adhesin to the tip fibrillum and the tip fibrillum to the rod, respectively [10]. The PapD chaperone and the PapC usher constitute the assembly machinery of the P pilus [11,12].

Type 1 pili are encoded by the *fim* gene cluster (*fimA–I*) and bind to mannose residues present in the human bladder (Fig. 1A) [4]. They have been shown to be important in the establishment of cystitis, or bladder infection, and have also been implicated in attachment to abiotic surfaces in a model biofilm system [13–16]. The type 1 pilus consists of a rod with dimensions similar to that of the P pilus joined to a short tip fibrillum ~ 10–19 nm in length [17]. The rod contains FimA subunits arranged in a tight, one-start, right-handed helix with a diameter of 6.9 nm, a pitch of 2.4 nm, and 3.4 subunits per turn [18]. Like the P pilus, it has a central cavity with radial channels that winds through the center of the rod. The tip fibrillum contains, from most proximal to most distal, FimF and FimG subunits and the mannose-specific FimH adhesin [17,19]. The FimC chaperone and the FimD usher constitute the assembly machinery of the type 1 pilus [20,21].

Two additional surface organelles, the capsular F1 antigen of *Y. pestis* and the haemagglutinating pilus of the human respiratory pathogen *H. influenzae*, highlight the structural diversity of fibers assembled by the chaperone–usher pathway. The capsular F1 antigen (Caf1) of *Y. pestis* is encoded by the *caf1* genes and expressed at 37 °C (Fig. 1A). It has been shown to enhance bacterial resistance to uptake by phagocytic cells and consists of thin fibrils of polymerized Caf1 subunits assembled by the Caf1M chaperone and the Caf1A usher [22–25]. The haemagglutinating pilus of *H. influenzae* is encoded by the *hif* gene cluster (*hifA–E*) and has been shown to facilitate colonization of the respiratory tract [26–28]. The pilus is a flexible rod that contains HifA subunits; the HifD subunit is localized near the distal end of the rod. The pilus is assembled by the HifB chaperone and the HifC usher [29]. Three-dimensional reconstruction of electron micrograph images indicates that the pilus rods are composed of three fibrils that wind around each other to form a three-start, left-handed helix 7.0 nm in diameter with three subunits per turn and a central channel, ~ 2.0 nm in diameter, that runs straight along its axis [30].

2. Overview of pilus fiber assembly

During assembly, pilus subunits enter the bacterial periplasm via the Sec translocation machinery [31] (Fig. 1). Subsequent assembly steps proceed in the absence of ATP or electrochemical gradients [32]. In the periplasm, each subunit interacts with a chaperone to form a stable chaperone–subunit complex. Interaction with the chaperone (i)

facilitates the efficient folding of the subunit, (ii) stabilizes it, preventing its aggregation and/or degradation, (iii) caps its interactive surfaces, inhibiting premature fiber formation, and (iv) primes it for assembly [33–39]. The chaperone is required for assembly—in its absence, subunits misfold and/or aggregate and are proteolytically degraded [40,41]. Thus in vivo, subunits are not found as soluble monomers but only in complex with a chaperone or as parts of the mature fiber. Chaperone–subunit complexes in the periplasm are targeted to the outer membrane usher [12]. In the cases of P and type 1 pili, the chaperone–adhesin complex binds most rapidly and tightly to the usher, forming an assembly-competent ternary complex and positioning the adhesin at the tip of the pilus [21]. The fiber then grows by the addition of subunits to the base. The usher is thought to facilitate chaperone uncapping to expose the interactive surfaces on the subunits that drive their polymerization. The usher forms a ring with a pore 2–3 nm in diameter, wide enough to allow passage to the extracellular milieu of a tip fibrillum and an unwound pilus rod but not of a rod in its final helical structure. Thus, it has been proposed that the pilus winds into its final quaternary structure once it has traversed the usher pore and is outside the cell [19,42].

3. The chaperone and donor strand complementation

The periplasmic chaperones of the chaperone–usher pathway share conserved structural features first revealed in the crystal structure of PapD and subsequently seen in the structures of three other family members [43–46] (Fig. 2). The two domains of each chaperone are oriented at an approximate right angle to each other to produce an L-shaped molecule. Each domain has an immunoglobulin-like (Ig) fold with seven primary β -strands (strands A–G) that form the staves of β -barrel. The Ig β -barrel consists of two β -sheets, the first made up of the B, E, and D strands and the second made up of the C, F, and G strands. The A strand is divided into the A1 and A2 segments—the A1 segment interacts with the B strand but then the A strand switches sheets such that the A2 segment interacts with the G strand. Alternating hydrophobic residues in the seven strands face the interior of the barrel and together constitute the hydrophobic core of the domain. The chaperone has an extended loop that connects the F and G strands of its N-terminal domain (the F₁ and G₁ strands, hence the F₁–G₁ loop). This F₁–G₁ loop lies at the end of one arm of the L and contains a conserved motif of solvent-exposed alternating hydrophobic residues that are involved in chaperone–subunit complex formation [47]. Chaperones can be classified based on the lengths of their F₁–G₁ loops—those with shorter loops (FGS chaperones, including PapD and FimC) are typically associated with rod-like pilus fibers, while those with longer loops (FGL chaperones, including Caf1M of *Y. pestis*) are typically associated with non-pilus fibers [1]. The chaperone G₁ strand runs from the F₁–G₁ loop toward the cleft

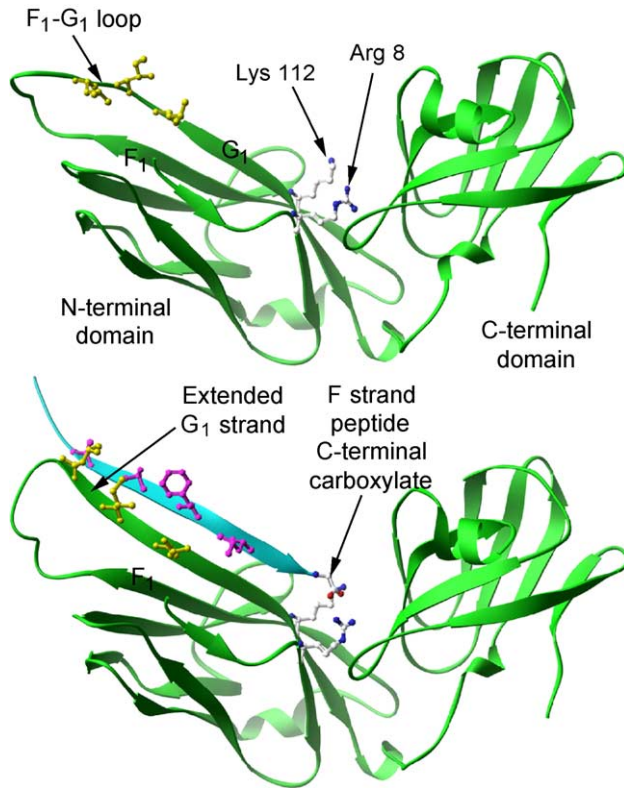


Fig. 2. The chaperone. Ribbon diagrams [58] of the crystal structures of PapD (upper panel) and of PapD bound to peptide corresponding to the C-terminus of the PapK subunit (lower panel), respectively, are shown. The N- and C-terminal domains, the F₁–G₁ loop, and the F₁ and G₁ strands of the chaperone (green) are labeled. The peptide interacts with the chaperone in the same manner as does the subunit F strand in a chaperone–subunit complex. The C-terminal carboxylate of the peptide is anchored between the conserved Arg 8 and Lys 112 residues in the chaperone cleft. The peptide (cyan) adopts a β -strand conformation as it zips along the chaperone G₁ strand, simultaneously inducing a conformational change in the chaperone F₁–G₁ loop, which becomes part of the G₁ strand as its alternating hydrophobic residues (yellow) intercalate between the alternating hydrophobic residues (magenta) of the peptide.

between the two domains of the chaperone, at the angle of the L. At the base of the cleft lie two conserved basic residues, a lysine in the G₁ strand and an arginine in the adjacent A₁ strand, that are essential for chaperone–subunit complex formation [34,41] (Fig. 2).

Subunits assembled by the chaperone–usher pathway share conserved structural features first revealed in the crystal structures of the FimC–FimH and PapD–PapK chaperone–subunit complexes [48–50] (Fig. 3). Most subunits consist of a single so-called pilin domain that interacts with the chaperone. Adhesin subunits like FimH generally have an additional domain, a receptor-binding domain that interacts with a specific receptor, N-terminal to their pilin domain. Each subunit pilin domain has an incomplete Ig fold. The pilin domain possesses strands A–F of the fold but lacks a C-terminal G strand, leaving its hydrophobic core incomplete and resulting in a groove or scar on the surface of the subunit. In the chaperone–subunit complex, residues

from the chaperone F₁–G₁ loop, including its alternating hydrophobic residues, have changed conformation to become part of the G₁ strand. In an interaction termed donor strand complementation, this elongated G₁ strand occupies the groove of the subunit (Fig. 3). The three alternating hydrophobic residues that were formerly exposed as part of the chaperone F₁–G₁ loop now project into the groove and

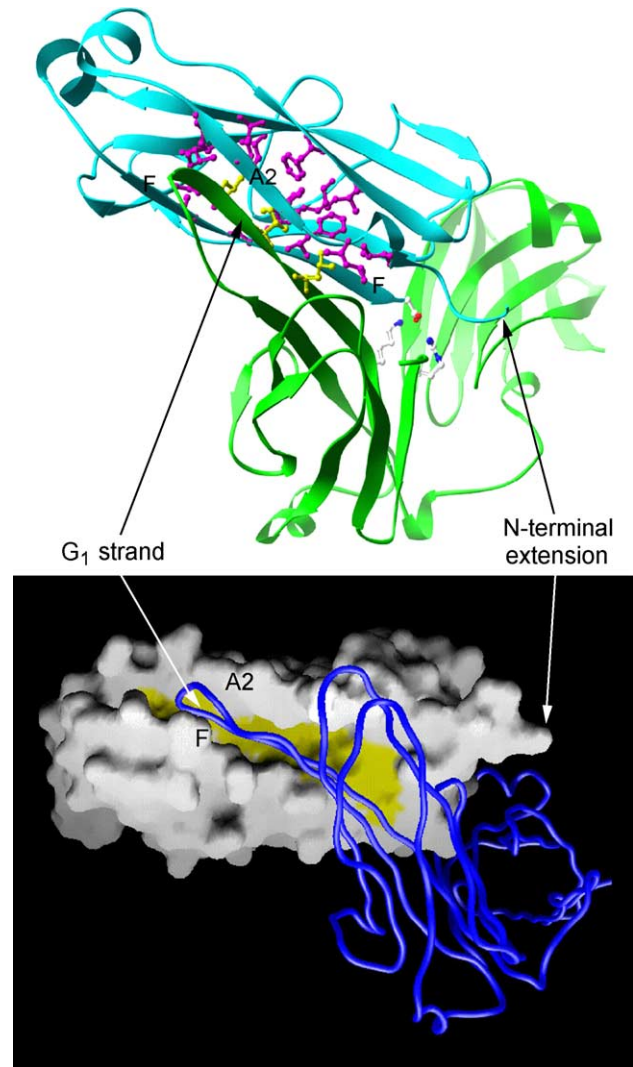


Fig. 3. Donor strand complementation. Two representations of the PapD–PapK chaperone–subunit complex are shown. In the upper panel, PapD is in green and PapK is in cyan. The chaperone G₁ strand lies between the subunit A₂ and F strands and completes its Ig fold. The alternating hydrophobic residues (yellow) of the G₁ strand contribute to the hydrophobic core (magenta) of the subunit. The clamping interaction in the chaperone cleft (red, white, and blue ball-and-stick as in Fig. 2) can be appreciated. In the crystal structure, residues 1–8 of PapK (the majority of the N-terminal extension) are disordered and not visible. The N-terminal extension label indicates residue 9 of PapK. In lower panel, PapD is depicted as a worm and PapK is shown in surface representation [59]. The view is looking into the subunit groove. The subunit hydrophobic core is in yellow. The exposed position of the subunit–N-terminal extension (residue 9 is indicated) in the complex and the wedging action of the chaperone, which holds the A and F strands away from each other at one end of the groove (right side of subunit as shown), can be appreciated.

complete the subunit hydrophobic core. The chaperone G₁ strand lies between the A and C-terminal F strands of the pilin and thus becomes the G strand of the pilin domain, completing its Ig fold. The fold is slightly atypical, however, since the chaperone G₁ strand runs parallel to the subunit F strand, whereas in a canonical Ig fold, the G strand runs in the opposite direction, anti-parallel to the F strand. The parallel orientation of the chaperone G₁ and subunit F strands in the complex places the very C-terminal carboxylate of the subunit, at the end of the F strand, at the base of the chaperone cleft. There, the positively charged side chains of the conserved lysine and arginine residues of the chaperone form two arms of a clamp, one on each side of the negatively charged subunit carboxylate (Figs. 2 and 3). This clamping interaction is thought to help anchor the subunit F strand and position it properly relative to the chaperone G₁ strand during subunit folding and complex formation. In a sense, then, the clamping interaction substitutes for the missing peptide bond that would normally link the F and G strands of an Ig fold.

Subunits do not fold efficiently in the absence of the chaperone. *In vitro* experiments indicated that the FimH subunit can adopt a native-like folded state in the absence of the chaperone under very carefully controlled conditions. However, even under the optimized conditions of these experiments, FimH folding was relatively slow and inefficient, and then FimH did not bind to the FimC chaperone efficiently and was not stable, but rather tended to aggregate [51]. The chaperone facilitates subunit folding by providing the subunit with a critical strand and elements of its hydrophobic core [37,38,48,52]. Two crystal structures of the PapD chaperone, bound to peptides corresponding to the F strands of the PapG and PapK subunits, respectively, shed light on the mechanisms of chaperone action during subunit folding [34,37] (Fig. 2). The peptides interact with the chaperone in the same manner as do subunit F strands in chaperone–subunit complexes. Each peptide binds as an extended β -strand that runs parallel to the chaperone G₁ strand. The C-terminal carboxylate of the peptide is clamped between the conserved basic residues in the chaperone cleft. The binding of the peptide induces the same conformational change in the chaperone F₁–G₁ loop that is seen in the chaperone–subunit complex. The F₁–G₁ loop becomes part of the G₁ strand as the C α backbone of the loop makes hydrogen bonds with the C α backbone of the peptide and the alternating hydrophobic residues of the loop intercalate between the alternating hydrophobic residues of the peptide—an interaction that has been termed a β -zipper. Taken together, the chaperone–peptide and chaperone–subunit structures suggest that during chaperone–subunit complex formation, the clamping interaction in the chaperone cleft positions the subunit F strand as it zips along the chaperone G₁ strand. The zipper interaction pulls the alternating hydrophobic residues of the chaperone F₁–G₁ loop into the G₁ strand, where their interaction with the hydrophobic residues of the F strand produces a hydrophobic bed that

facilitates the proper collapse of the subunit core. The zipper interaction also likely simultaneously facilitates β -sheet formation in the subunit [37]. It has been shown that a preformed β -strand can nucleate the formation of a β -sheet [53]. Thus, the adoption of β -strand conformation by the subunit F strand as it zips along the chaperone G₁ strand likely facilitates the adoption of β -strand conformation by the subunit C strand on its other side, yielding one of the two subunit β -sheets. Since the β -strands of the subunit include its hydrophobic core residues, the facilitation of β -strand formation and the promotion of proper hydrophobic core collapse would reinforce each other. The facilitation by the chaperone of both processes would therefore favor rapid and efficient subunit folding. By donor strand complementation, then, the chaperone provides the subunit with steric information required for its efficient folding. The chaperone then remains bound to the subunit, preventing its aggregation by stabilizing it in the chaperone–subunit complex.

4. Donor strand exchange

Mutagenesis studies indicated that the residues in the subunit F strand that line one side of the groove participate in subunit–subunit interactions, identifying the groove as a critical surface in subunit polymerization [37]. In the chaperone–subunit complex, therefore, the chaperone G₁ donor strand transiently caps the subunit by occupying and shielding its interactive groove. Upstream of the A strand that forms the other side of the groove, most subunits have an N-terminal extension (roughly residues 1–11 of the mature protein in the case of the PapK subunit, for example) that in the chaperone–subunit complex is disordered and does not contribute to the subunit Ig fold [49]. Like the chaperone G₁ strand, the N-terminal extension contains a conserved motif of alternating hydrophobic residues. As with the residues of the subunit F strand, mutagenesis studies indicated that these alternating hydrophobic residues participate in subunit–subunit interactions, identifying the N-terminal extension as a second critical element in subunit polymerization [37]. During fiber assembly, then, subunit incorporation occurs via a donor strand exchange mechanism, in which the N-terminal extension of one subunit replaces the chaperone G₁ strand in the groove of the neighboring subunit [39,46,48,49] (Fig. 4). The adhesin at the tip of a pilus fiber lacks an N-terminal extension—it has instead its N-terminal receptor-binding domain—but still has a groove that can interact with the N-terminal extension of an adjacent subunit. Donor strand exchange therefore occurs such that the N-terminal extension of the incoming subunit occupies the groove of the most recently incorporated subunit, producing a mature organelle in which each subunit contributes its N-terminal extension to complete the Ig fold of its more distal neighbor.

Subunit–subunit interactions in the fiber are even more stable than chaperone–subunit interactions. For example, subunit–subunit complexes and whole pilus fibers, but not

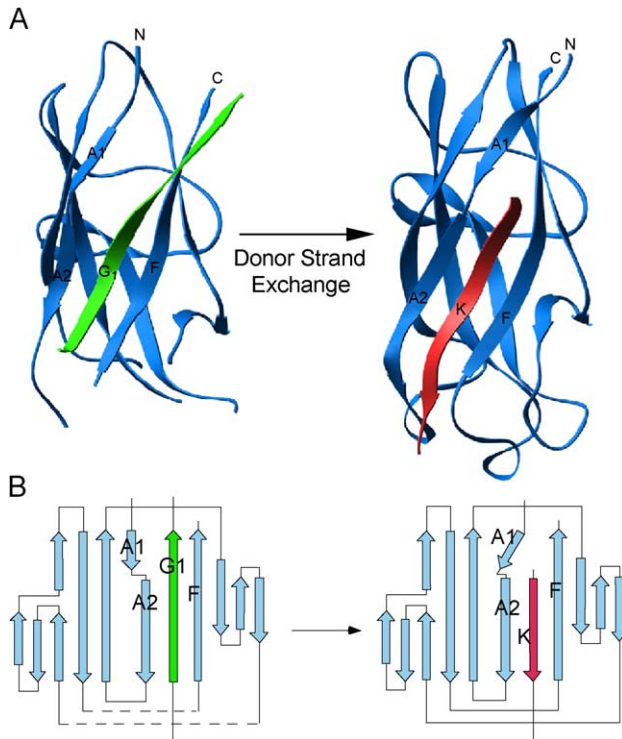


Fig. 4. Donor strand exchange. (A) A three-dimensional view of donor strand exchange. The PapD–PapE chaperone–subunit complex (left, only the G₁ strand (green) of the chaperone is shown) and a complex of the PapE subunit (blue) bound to a peptide corresponding to the N-terminal extension (residues 1–11) of PapK (red) are shown. Strands are labeled. Note the reversal in orientation and shift in register of the complementing strands upon donor strand exchange. Note also the positions of the labeled N- and C-termini (corresponding to the beginning of the A strand and the end of the F strand, respectively, on either side of the groove) of the subunit in each complex. The displacement of the chaperone allows the A and F strands to move together at this end of the groove, closing it and sealing the N-terminal extension in place. In the chaperone–subunit complex, the loops at the end of the subunit away from its N- and C-termini are (bottom of view as shown) disordered. The adoption of a more compact, ordered state by the subunit after donor strand exchange can be appreciated. (B) A topological view. In this diagram, arrows represent strands. The donated strands by the chaperone (the G₁ strand in the diagram at left) or by the subunit next in line in the assembly process (the N-terminal extension of that subunit in the diagram at right) are shown in the same color-coding as in A.

chaperone–subunit complexes, resist dissociation in 2% SDS at room temperature [37,54]. In addition, *in vitro* studies in the absence of other factors reveal that an excess of the PapK subunit, whose N-terminal extension binds in the groove of the PapE subunit, displaces the chaperone from a PapD–PapE complex. However, PapK will not displace a peptide corresponding to the N-terminal extension of PapK from the PapE subunit groove [39]. These results indicate that the subunit is in an activated state when bound to the chaperone and only attains its “ground” state after donor strand exchange when it is bound to the N-terminal extension of its neighbor. The chaperone thus primes the subunit for donor strand exchange.

Structural studies of the P pilus and of the *Y. pestis* CafI fiber systems, by providing views of subunits before and

after donor strand exchange, reveal the mechanism of subunit priming by the chaperone [39,46]. In the P pilus system, two crystal structures of the PapE pilus subunit, in complex with the PapD chaperone and in complex with a peptide corresponding to the N-terminal extension of the PapK subunit, respectively, were determined [39] (Fig. 4). In the PapD–PapE and other chaperone–subunit complexes, as the chaperone G₁ strand runs the length of the subunit, it gradually curves away from the centerline of the subunit groove, pulling with it the parallel subunit F strand and holding it away from the subunit A strand on the other side of the groove (Figs. 3 and 4). The chaperone acts as a wedge between the ends of the A and F strands, holding one end of the groove in a relatively open conformation and preventing the compact packing of the underlying hydrophobic core residues [39,46]. In the subunit–N-terminal-extension complex, the N-terminal extension has replaced the chaperone G₁ strand and occupies the subunit groove. Whereas the chaperone G₁ strand runs parallel to the subunit F strand in the chaperone–subunit complex, in the subunit–N-terminal-extension complex the N-terminal extension runs in the opposite direction, anti-parallel to the subunit F strand, to produce a perfectly canonical Ig fold in the subunit. The alternating hydrophobic residues of the N-terminal extension replace those of the G₁ strand in the subunit groove, but to produce a snug fit they are shifted in register, one position further from the open end of the groove. The displacement of the chaperone and the subsequent orientation and register of the N-terminal extension in the groove trigger a conformational change in the subunit. The removal of the chaperone wedge allows the ends of the subunit A and F strands to move together. The underlying hydrophobic core residues pack more tightly and the groove closes, sealing the N-terminal extension in place as the subunit adopts a more ordered and compact overall state [46] (Fig. 4).

In the CafI system, the structure of a chaperone–subunit–subunit CafIM–CafI–CafI ternary complex was determined [46]. The CafIM chaperone, an FGL chaperone with its characteristic long F₁–G₁ loop, is bound to one CafI subunit, which in turn contributes its N-terminal extension to interact with the second subunit. The structure of the complex thus provides views of both chaperone–subunit and subunit–subunit interactions. The chaperone–subunit and subunit–subunit interactions exhibit the characteristic features of donor strand complementation and exchange, including the maintenance of the groove in an open conformation by the wedging action of the chaperone and the reversal in complementing strand orientation upon donor strand exchange. The groove of the CafI subunit is longer than those of the subunits from P pili and type 1 pili, and hence the chaperone G₁ strand, derived in part from the long F₁–G₁ loop, is correspondingly longer, contributing five alternating residues to fill the subunit groove [23]. Upon donor strand exchange, the N-terminal extension likewise contributes five alternating residues to the groove, but unlike

the case of the P pilus system, these residues are in the same register as those of the chaperone G₁ strand. However, after donor strand exchange the groove is shallower and narrower than it is in the chaperone–subunit complex, and to fit snugly in the groove the alternating residues from the N-terminal extension donor strand are correspondingly smaller than those of the chaperone G₁ strand. The shallow character of the groove results from the removal of the chaperone wedge, whose relatively bulky alternating G₁ strand residues penetrate more deeply into the subunit hydrophobic core and hold the two sides of the groove away from each other. The displacement of the chaperone allows the A and F strands on either side of the groove to move together and permits the tight packing of the underlying subunit hydrophobic core residues to yield a compactly packed molecule.

The chaperone thus primes the subunit for donor strand exchange by stabilizing it in an activated conformation. The chaperone facilitates the folding of the subunit, but, by holding the groove open and preventing the compact packing of the subunit hydrophobic core, it traps the subunit in an activated folding transition state [39,46]. Thus, chaperone catalysis of subunit folding permits the storage of folding energy that subsequently drives fiber formation [46]. The displacement of the chaperone by the N-terminal extension releases this stored energy, and the subunit completes its folding process, undergoing a topological transition from a loosely packed, non-canonical Ig fold to its final compact, canonical Ig fold in context of the fiber [39,46]. The characteristic stability of the fiber then derives from the contribution by each subunit of an integral strand to the fold of its neighbor.

5. Donor strand exchange at the usher

Donor strand exchange occurs very rapidly *in vivo* but only relatively slowly and inefficiently *in vitro* in the absence of the usher [32,39]. This suggests that while the chaperone primes the subunit for donor strand exchange, active subunit uncapping at the usher is required for efficient fiber formation. Fiber assembly at the usher includes the targeting of chaperone–adhesin and other chaperone–subunit complexes to the usher, the uncapping of subunits and the facilitation of donor strand exchange, and the translocation of the growing fiber through the usher pore. Recent studies have only begun to reveal the mechanisms that drive these processes.

Experimental evidence indicates that the chaperone plays a role in donor strand exchange at the usher. Mutations in two conserved surface-exposed residues of the PapD chaperone—Thr 53 and Arg 68—reduce pilus assembly without affecting chaperone–adhesin complex formation or the interaction of this complex with the usher [1,47]. These results indicate that the chaperone is involved in a critical step in fiber assembly after the formation of the chaperone–adhesin–usher complex. Additional studies point to a role

for the chaperone in incorporation of subunits into the fiber [55]. Further work will elucidate the details of chaperone function at the usher.

The usher has been modeled as an outer-membrane β -barrel, with its larger inter-strand loops facing the periplasm. The periplasmic loops are thought to include both a region near the N-terminus as well as the C-terminal region of the protein, both of which contain a single conserved disulfide bond [56]. In both P pilus assembly and type 1 pilus assembly, the chaperone–adhesin complex initiates fiber formation by binding to the usher [12,21]. In the case of the type 1 pilus, it has been shown that both the receptor-binding and pilin domains of the FimH adhesin interact with the FimD usher. It was proposed that the receptor-binding domain of FimH inserts into the pore, while the pilin domain, bound to the chaperone, straddles the periplasmic surface of FimD [55]. The binding of the FimC–FimH chaperone–adhesin complex to the usher induces a conformational change in FimD and yields a stable FimC–FimH–FimD chaperone–adhesin–usher ternary complex that has been successfully isolated [21]. Limited proteolysis of this complex indicated that FimC–FimH interacts with the C-terminal half of FimD. Expression of the subunit FimG with FimC–FimH–FimD permitted the isolation of a FimC–FimG–FimH–FimD chaperone–subunit–adhesin–usher complex in which FimG was present in multiple copies. Electron microscopic visualization of the complex revealed a fiber emerging from one end of the usher pore [19]. The presence of the FimC chaperone in this complex suggests that the chaperone of the most recently incorporated subunit remains bound at the base of the pilus, on the periplasmic side of the usher, until it is displaced by the incoming chaperone–subunit complex during fiber assembly [17,19]. The additional expression of the FimF subunit and the FimA rod subunit in this system then permitted the isolation of a complex that included the chaperone and an intact type 1 pilus, including tip fibrillum and rod, with an usher at its base.

Recent studies indicate that an N-terminal periplasmic region of FimD also binds to chaperone–subunit complexes [57]. Likewise, deletion studies of the PapC usher from the P pilus system indicate that the PapD–PapG chaperone–adhesin complex binds to PapC truncations that lack significant portions of their C-terminal region [56]. However, the PapC C-terminal region is required for pilus assembly, since these PapC truncations do not assemble fibers *in vivo*. These results suggest that the usher contains two binding sites for chaperone–subunit complexes, one in its N-terminal region for chaperone–subunit complex targeting and one in its C-terminal region for subunit assembly [56].

6. Conclusions

Recent advances in the structural biology of pilus biogenesis have elucidated many aspects of the mechanism of

pilus biogenesis. Pilus subunits all have a missing secondary structure, the G strand, and the hydrophobic groove resulting from such a structural truncation is the site for both chaperone binding and subunit–subunit interaction. The structures of chaperone–subunit and subunit–subunit complexes have revealed that the chaperone provides the missing secondary structural element through a mechanism termed “donor strand complementation” and that the secondary structure provided by the chaperone (the G1 strand) is exchanged for the N-terminal extension of the subunit next in the assembly line during pilus assembly through a mechanism termed “donor-strand exchange”. The structures also reveal that the energy that drives the process is likely to be provided by the conformational transition from chaperone-bound to subunit-bound that all subunits undergo during donor strand exchange. Indeed, in the periplasm, subunits appear to be maintained in a semi-unfolded state stabilized by the interaction with the chaperone. Once dissociation from the chaperone occurs, donor strand exchange occurs and the subunits “snap” into a folded state: the energy released by this process is likely to be sufficient to drive the polymerization.

Crucial to the process is the usher. The usher is where the process of donor strand exchange occurs and very little is known about the mechanisms whereby the usher destabilizes chaperone–subunit complexes to trigger chaperone release, and stabilizes the uncapped state of subunits once the chaperone has dissociated. The structure of the usher and of the larger complexes that it forms with chaperone–subunit complexes will no doubt provide answers to these questions and this is expected to be the next challenge in the field.

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