Chaperone-assisted pilus assembly and bacterial attachment
Frederic G Sauer*, Michelle Barnhart*, Devapriya Choudhury‡, Stefan D Knight‡, Gabriel Waksman† and Scott J Hultgren*§

Bacterial pili assembled by the chaperone-usher pathway can mediate microbial attachment, an early step in the establishment of an infection, by binding specifically to sugars present in host tissues. Recent work has begun to reveal the structural basis both of chaperone function in the biogenesis of these pili and of bacterial attachment.

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Abbreviations
dsc donor-strand-complemented
UTI urinary tract infection

Introduction
Gram-negative bacteria assemble a diverse array of complex surface organelles that play roles in many cellular processes, including motility, DNA uptake, secretion of virulence factors and attachment to and invasion of host tissues. The construction of these organelles requires that the bacterium coordinate the folding, secretion and ordered assembly of multiple distinct subunit proteins. Pili are fibrous surface organelles that can mediate attachment to host tissues, a critical early step in the development of a variety of diseases [1]. Gram-negative bacteria assemble pili by various pathways, including the chaperone-usher pathway, the type IV pilus assembly pathway, the alternative chaperone-usher pathway and the type IV secretion pathway [2–9]. Pili assembled by the chaperone-usher pathway contain adhesins that bind specifically to sugars present in host tissues. This review will focus on recent advances in our understanding of the structural basis both of the biogenesis of pili by the chaperone-usher pathway and of the attachment to host tissues mediated by these fibers. This work has begun to reveal the molecular mechanisms of both pilus biogenesis and organelle assembly in general. The insight gained into these critical pathogenic events promises to aid the development of new methods to prevent and treat bacterial diseases.

Organelle assembly by the chaperone-usher pathway
The chaperone-usher pathway participates in the assembly of more than 30 pilus and nonpilus surface organelles [2–4] (Table 1). Pili assembled via this pathway vary subtly in their gross structures. For example, the P pilus, encoded by the 11 genes of the pap (papA–papK) gene cluster, found in many uropathogenic strains of Escherichia coli, is a composite fiber — a thick rod with a thinner tip fibrillum at its distal end [10,11]. The rod has a diameter of 7 nm, with a hollow core, and is composed of primarily PapA subunits wound in a tight right-handed helix; the tip fibrillum consists of primarily PapE subunits wound in an open-helical conformation [11–13]. The PapG adhesin, which binds to gal(α1→4)gal-containing sugars found in the human kidney and is necessary for the development of pyelonephritis in a monkey model, is located at the tip of the fibrillum [11,14,15]. Two adaptor subunits, PapF and PapK, are thought to link the adhesin to the tip fibrillum and the tip fibrillum to the rod, respectively [16]. The type 1 pilus, encoded by the fim gene cluster (fimA–fimH), has a similar composite structure, but its tip fibrillum is short and stubby. The rod is composed of predominantly FimA subunits wound in a tight right-handed helix; the tip fibrillum contains the mannose-specific adhesin FimH, as well as FimG. FimF is thought to link the tip fibrillum to the rod [10,17,18,19**]. Analysis of the mannose-binding activity of fragmented type 1 pili suggested that FimH is also intercalated in the rod, but with its mannose-binding activity buried. Breakage of the pili at these sites would then expose the mannose-binding activity of these FimH molecules [20]. Type 1 pili are found in most E. coli strains, including both uropathogenic and commensal strains, as well as throughout the Enterobacteriaceae family, and have been shown to play a critical role in the pathogenesis of cystitis [21–23]. Hif pili, encoded by the hif gene cluster (hifA–hifE), found in pathogenic Haemophilus influenzae strains, have rods 6–7 nm in diameter and a short, thinner tip differentiation. The rods have a cross-over repeat consistent with a double-stranded right-handed helical architecture. The rod is composed of primarily HifA, whereas the tip contains HifD and HifE [24–28]. Inhibition of hemagglutination by anti-serum raised against HifE indicates that this protein mediates attachment to human cells [28].

The biogenesis of each organelle assembled by the chaperone-usher pathway involves two dedicated proteins, a periplasmic chaperone and an outer membrane usher (Figure 1). Subunits enter the periplasm through the Sec apparatus. The chaperone — PapD in the pap system, FimC in the fim system, HifB in the hif system — then interacts with each newly translocated subunit individually, facilitating its release from the cytoplasmic membrane in a process that may be driven by the folding of the subunit directly on the chaperone template [27,29–32,33*,34**]. The chaperone remains bound to the folded subunit, forming a stable chaperone–subunit complex. In the absence of the chaperone, subunits are degraded. The formation of the chaperone–subunit complex thus serves to stabilize the...
Chaperone-assisted pilus assembly and bacterial attachment Sauer et al. 549

Figure 1

Pilus biogenesis. Single letters indicate the corresponding pap gene. (i) Pilus subunits enter the periplasm through the Sec machinery (YEG). In the absence of the chaperone, subunits misfold, aggregate and are degraded (ii). The PapD chaperone binds to nascent subunits and is thought to facilitate their folding by donating its G1 strand to complete their Ig-like folds in a mechanism termed donor strand complementation (iii). Donor strand complementation simultaneously stabilizes the subunit and caps its interactive groove. The soluble chaperone–subunit complexes are targeted to the PapC outer membrane usher. The PapD–PapG chaperone–adhesin complex binds most rapidly and tightly to the usher (iv), initiating pilus assembly and ensuring that the adhesin, which binds gal(α1→4)gal sugars, is at the tip of the pilus, which grows from the base. Incorporation of subsequent subunits is thought to occur by donor strand exchange, in which the N-terminal extension of a subunit displaces the chaperone G1 strand to occupy the groove of the subunit most recently incorporated in the pilus (v). The pilus is thought to move through the usher pore in a linear manner and adopt its final quaternary structure once outside the cell (vi).

Structural basis for chaperone function: donor strand complementation

The crystal structure of the PapD chaperone and the NMR structure of the FimC chaperone have been solved [42,43•]. These chaperones consist of two immunoglobulin (Ig)-like domains, with the overall shape of a boomerang. A conserved interdomain salt bridge maintains the relative orientation of the two domains [4,43•,44]. The conservation of the hydrophobic core residues among chaperones in the PapD superfamily indicates that they most probably share the same overall structure [4,43•]. Two crystal structures of PapD in complex with peptides derived from the C terminus of either the PapG adhesin or the PapK subunit revealed that the peptides bound in an extended parallel β-sheet conformation along the G1 β strand of the N-terminal domain of PapD [32,37]. The C-terminal carboxylate of each peptide was anchored in the cleft of the chaperone by interactions with the conserved Arg8 and Lys112 residues of the latter molecule. Alternating conserved hydrophobic residues in the chaperone G1 β strand and in the peptide together formed a large hydrophobic area on the surface of the chaperone–peptide complex that was suggested to nucleate subunit folding on the chaperone [32]. Subsequent transverse relaxation-optimized spectroscopy (TROSY) NMR measurements of the FimC–FimH chaperone–adhesin domain mapped the surface of FimC involved in the interaction with FimH and indicated that it extended beyond the chaperone G1 β strand, but was essentially limited to the N-terminal domain [45••].

Recently, the crystal structures of the FimC–FimH complex and the PapD–PapK complex were solved [46••,47••]. The FimH adhesin consists of two domains, an N-terminal receptor-binding, or lectin, domain and a C-terminal pilin domain.
Both the single domain of PapK and the pilin domain of FimH have Ig folds that lack the Ig fold C-terminal seventh β strand (strand G). The conservation of their hydrophobic core residues, as well as experimental evidence from mutagenesis studies, indicates that subunits assembled into pili by the chaperone-usher pathway share this C-terminally truncated Ig fold [2,36]. The lack of the C-terminal β strand produces a cleft or groove on the surface of the subunit and exposes the hydrophobic core of the domain. In an interaction termed ‘donor strand complementation’, the G1 β strand of the chaperone occupies the groove of the subunit, completing its Ig fold by providing the missing seventh β strand (Figure 2) [46••,47••]. The G1 β strand lies between the A′′ (or A2) strand and the C-terminal F strand (corresponding to the C-terminal peptides crystallized in complex with PapD) of the subunit (Figure 3a). The interaction between the chaperone G1 β strand and the subunit F strand is essentially the same as that seen in the PapD–peptide complex structures [32,37]. The G1 β strand thus completes the Ig fold of the subunit in a noncanonical manner, as it runs parallel to the F strand, rather than antiparallel, as it would in a canonical Ig domain. The donor strand complementation interaction presumably stabilizes the subunit by shielding its hydrophobic core; indeed, the conserved alternating hydrophobic residues in the G1 β strand of the chaperone become part of the hydrophobic core of the subunit (Figure 2). Mutagenesis and biochemical experiments have implicated the groove of the subunit in subunit–subunit interactions [35,36]. Thus, donor strand complementation also prevents premature subunit polymerization by capping the interactive groove.

**Donor strand exchange**

Pilus subunits have an N-terminal extension (residues 1–13 in PapK) that does not contribute to the fold of the subunit, but rather is generally disordered in the PapD–PapK crystal structure [47••]. The N-terminal

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### Table 1

**Bacterial surface organelles assembled via the chaperone-usher pathway**.

<table>
<thead>
<tr>
<th>Organelle</th>
<th>Organism</th>
<th>Chaperone</th>
<th>Usher</th>
<th>Disease</th>
</tr>
</thead>
<tbody>
<tr>
<td><strong>Fibers</strong></td>
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<tr>
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<td><em>E. coli</em></td>
<td>PapD</td>
<td>PapC</td>
<td>Pyelonephritis, cystitis</td>
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<tr>
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<td><em>E. coli</em></td>
<td>PrsD</td>
<td>PrsC</td>
<td>Cystitis?</td>
</tr>
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<td><em>E. coli</em>, <em>Salmonella</em> spp., <em>Klebsiella pneumoniae</em></td>
<td>FimC</td>
<td>FimD</td>
<td>Cystitis</td>
</tr>
<tr>
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<td><em>E. coli</em></td>
<td>FocC</td>
<td>FocD</td>
<td>Cystitis?</td>
</tr>
<tr>
<td>S pilus</td>
<td><em>E. coli</em></td>
<td>SfaE</td>
<td>SfaF</td>
<td>UTI, NBM</td>
</tr>
<tr>
<td>Hif pilus</td>
<td><em>H. influenzae</em></td>
<td>HifB</td>
<td>HifC</td>
<td>Otitis media, meningitis</td>
</tr>
<tr>
<td>Haf pilus</td>
<td><em>H. influenzae</em> biogroup aegyptius</td>
<td>HafB</td>
<td>HafC</td>
<td>Brazilian purpuric fever</td>
</tr>
<tr>
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<td>FimC (FimA)</td>
<td>Whooping cough</td>
</tr>
<tr>
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<td><em>S. typhimurium</em></td>
<td>PeID</td>
<td>PeF</td>
<td>Gastroenteritis, salmonellosis</td>
</tr>
<tr>
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<td><em>S. typhimurium</em></td>
<td>LptB</td>
<td>LptC</td>
<td>Gastroenteritis?, salmonellosis?</td>
</tr>
<tr>
<td>MR/P pilus</td>
<td><em>Proteus mirabilis</em></td>
<td>MrpD</td>
<td>MrpC</td>
<td>Nosocomial UTI</td>
</tr>
<tr>
<td>PMF pilus</td>
<td><em>P. mirabilis</em></td>
<td>PmID</td>
<td>PmF</td>
<td>Nosocomial UTI</td>
</tr>
<tr>
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<td>AftC</td>
<td>UTI</td>
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<td>AfrD</td>
<td>Diarrhea in rabbits</td>
</tr>
<tr>
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<td>FanD</td>
<td>Neonatal diarrhea in calves, lambs, piglets</td>
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<td>FaeD</td>
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<td>FasD</td>
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<td>F17papC</td>
<td>Diarrhea in piglets</td>
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<tr>
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<td>MrkC</td>
<td>Pneumonia</td>
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<td>NfaC</td>
<td>UTI, NBM</td>
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<td>AfaC</td>
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<td>DraE</td>
<td>DraD</td>
<td>UTI, diarrhea</td>
</tr>
<tr>
<td>M</td>
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<td>BmaC</td>
<td>Pyelonephritis</td>
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<tr>
<td><strong>Atypical structures</strong></td>
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<td>Cs3-1</td>
<td>Cs3-2</td>
<td>Traveler’s diarrhea</td>
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<td>CS3A1 pilus</td>
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<td>ClpE</td>
<td>ClpD</td>
<td>Diarrhea</td>
</tr>
<tr>
<td>CS6 pilus</td>
<td><em>E. coli</em></td>
<td>CssC</td>
<td>CssD?</td>
<td>Diarrhea</td>
</tr>
<tr>
<td>AAF/1</td>
<td><em>E. coli</em></td>
<td>AggD</td>
<td>AggC</td>
<td>Diarrhea</td>
</tr>
<tr>
<td>Sef</td>
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<td>SefB</td>
<td>SefC</td>
<td>Gastroenteritis, salmonellosis</td>
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<td>CalA</td>
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<td>PsbC</td>
<td>Plague</td>
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<td>MyfB</td>
<td>MyfC</td>
<td>Enterocolitis</td>
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<td>?</td>
<td><em>E. coli</em></td>
<td>RalE</td>
<td>RalD</td>
<td>Diarrhea in rabbits</td>
</tr>
</tbody>
</table>

*Adapted from Table 1 in [3]. See [3,4,49] for further details. NBM, newborn meningitis.
extension has a conserved motif of alternating hydrophobic residues reminiscent of that found in the G₁ β strand of the chaperone. Thus, it has been proposed that, during pilus biogenesis, the N-terminal extension of one subunit displaces the G₁ β strand of the chaperone bound to the subunit most recently incorporated into the pilus (Figure 3b) [46**,47**]. Modeling of a pilus rod on the basis of its known helical structure and dimensions indicates that the N-terminal strand would run antiparallel, rather than parallel, as the chaperone G₁ β strand does in the case

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**Figure 2**

Donor strand complementation in the PapD–PapK structure. A stereo ribbon diagram of the PapD chaperone (cyan) in complex with the PapK subunit (gray). The chaperone G₁ strand completes the Ig fold of the subunit, making β-strand interactions with the A₂ and F strands of PapK (see also Figure 3). The alternating hydrophobic residues (red) of the G₁ strand interact with the hydrophobic core residues (yellow) of PapK. The conserved Arg8 and Lys112 residues of the chaperone anchor the C-terminal carboxylate group of the subunit in the cleft of the chaperone. The N-terminal extension of PapK (upstream of the gray coil in the foreground) is disordered and not visible in the structure. The FimH adhesin has a receptor-binding domain here, rather than an N-terminal extension (see also Figures 3 and 4), consistent with its location at the tip of the pilus.

**Figure 3**

Topology diagrams. Dashes indicate additional polypeptide not shown. (a) Donor strand complementation. The chaperone donates its G₁ strand (red) to complete the Ig fold of the subunit (white). The fold is noncanonical, as the G₁ strand runs parallel to the F strand. The N-terminal extension is shown as a blue strand. (b) After donor strand exchange. The N-terminal extension of one subunit now completes the Ig fold of its neighbor in a canonical manner, as the N-terminal extension runs antiparallel to the F strand. (c) Donor-strand-complemented FimH (dscFimH). DscFimH was constructed by fusing the N-terminal extension of FimG (blue), which is predicted to complete the fold of FimH in the pilus, to the C terminus of FimH with a four amino acid linker (green). Unlike wild-type FimH, dscFimH is stable in vivo in the absence of the chaperone.
of donor strand complementation, to the F strand of the subunit whose fold it completes. Thus, this donor strand exchange mechanism of pilus assembly would produce a mature organelle in which each subunit contributes a strand to complete the Ig fold of its neighbor in a perfectly canonical manner. This switch in directionality of the donor strand during donor strand exchange may be part of the mechanism that ensures that the transient chaperone–subunit interactions are less stable than the more permanent subunit–subunit interactions [48].

Chaperone-assisted subunit folding
It has been hypothesized that one function of the chaperone is to facilitate the folding of the pilus subunits [32,49]. Alternatively, it is possible that, during pilus biogenesis, each subunit folds on its own in the periplasm and then binds to the chaperone. In this scenario, fully folded subunits might also interact with other fully folded subunits in the periplasm, setting up a competition between productive chaperone–subunit interactions and potentially nonproductive periplasmic subunit–subunit interactions. Alternatively, the chaperone could directly facilitate subunit folding by providing missing steric information in the form of its G1 β strand. In this model, the chaperone first recognizes a portion of the non-native subunit, perhaps the C-terminal carboxylate group and C-terminal F strand, in an interaction similar to that seen in the chaperone–peptide complexes [32,37]. The subunit would then fold on the chaperone template to produce the fully formed chaperone–subunit complex [32]. The chaperone would thus couple the folding of the subunit to the capping of its interactive groove, ensuring that the groove is never free to interact nonproductively with other subunits [32,49]. Recent in vitro studies indicate that wild-type FimH denatured in urea does not refold upon dilution at the conditions tested. In contrast, dilution of the same material in the presence of the FimC chaperone led to the productive folding of FimH to yield a FimC–FimH complex [34••]. A donor-strand-complemented version of FimH (dscFimH) was then constructed by fusing the N-terminal extension of FimG, which is predicted to complete the fold of FimH in the mature type 1 pilus, to the C terminus of FimH to yield a single molecule that should form a completely canonical Ig fold (Figure 3c). Unlike wild-type FimH, dscFimH folded into a native structure in vivo and refolded into a native mannose-binding conformation in the urea dilution assay [34••]. These results indicate that the missing steric information required for subunit folding at these conditions can be provided in cis and suggest that the chaperone does indeed facilitate subunit folding by donor strand complementation.

It has been shown that the formation of β-strand secondary structure is context-dependent, rather than sequence-dependent [50]. The G1 β strand may provide the appropriate context for the formation of the G1FC β sheet, which forms a portion of the Ig fold of the subunit in the complex (Figure 3a). The interaction between the subunit C-terminal carboxylate group and the conserved arginine and lysine residues in the chaperone cleft may anchor the subunit C-terminal F strand to facilitate formation of this β sheet (Figure 2). Such an interaction would simultaneously position the subunit to allow the alternating hydrophobic residues of the chaperone G1 β strand to contribute to the appropriate formation of the hydrophobic core of the subunit. This model suggests that the steric information required for subunit folding resides not in a single amino acid chain, but rather in two distinct polypeptides [32,34••,49].

FimH receptor binding: structural details
The FimH adhesin of type 1 pili can bind both monomannose and oligomannose moieties associated with a variety of substrates. Naturally occurring E. coli FimH alleles bind oligomannose moieties with similar affinities, but can be classified on the basis of their affinity for monomannose. Those that bind monomannose with low affinity are generally associated with commensal intestinal strains, those that bind with high affinity with uropathogenic strains [51,52•]. The former phenotype is thought to provide an advantage in colonizing the oral cavity and thence the intestinal tract, the latter in colonizing the urinary tract. Thus, it has been proposed that the FimH polymorphism arises from selection mechanisms that balance these two advantages [52•]. Expression of type 1 pili has been shown to enhance the virulence of uropathogenic E. coli in a mouse urinary tract infection (UTI) model by promoting both bacterial survival and the host inflammatory response to infection [22]. A clinical study revealed that the disease associated with UTI caused by type-1-expressing E. coli was more severe than that associated with type-1-negative strains of the same serotype [22] in children. High-resolution electron microscopy images show that type 1 pili mediate attachment to the uroplakin-coated surface of bladder epithelial cells in mice. Infection by type-1-expressing E. coli, but not by an isogenic fimH− strain, leads to the exfoliation of these uroplakin-coated cells, presumably a host defense mechanism designed to eliminate the infection. Some bacteria, however, are able to persist in the host by invading the now exposed underlying cells, a mechanism that has been proposed to contribute to the frequent recurrence of UTIs in many patients [53••]. Recent results indicate that FimH mediates the bacterial invasion of human bladder epithelial cells [54••]. FimH has also been shown to mediate attachment to mast cells and macrophages ([55–57]; see also Update). In the latter case, the attachment allows the bacteria to bypass the normal macrophage-killing pathway and to survive intracellularly for at least 24 hr [56]. FimH also binds to laminin and collagen in vitro. Although collagen lacks terminal mannose residues, FimH binding was inhibited by mannosic [58]. Finally, type 1 pili have been implicated in bacterial biofilm formation in one model system [59*].

A putative mannose-binding pocket has been identified at the tip of the receptor-binding domain of FimH. A
molecule of cyclohexylbutanoyl-N-hydroxyethyl-D-glu-camide (C-HEGA), which is not a known inhibitor of FimH–mannose binding, but which was required for the production of useful FimC–FimH crystals, was found bound near the tip of the receptor-binding domain of FimH in the crystal structure of the complex. Amino acids Phe1, Ile13, Asn46, Asp47, Tyr48, Ile52, Asp54, Gln133, Asn135, Tyr137, Asn138, Asp140 and Phe142 line this putative mannose-binding pocket and give it an overall negative charge (Figure 4) [46**]. The pocket is large enough to completely bury a monomannose residue, but cannot accommodate anything larger. The tight binding of oligomannose moieties is thus expected to involve additional contacts to subsites in the vicinity of the mannose-binding pocket. A mutation of Pro49 near the pocket abolishes monomannose binding and significantly reduces oligomannose binding as well [60**]. A double mutation of Asn136 and Tyr137 inhibits hemagglutination of guinea pig erythrocytes [61], an assay that measures mannose-binding activity. Mutations that increase the ability of FimH to bind mannose map to regions in the receptor-binding domain away from the putative binding pocket. It has been proposed that these mutations alter the conformational stability of the protein loops in the pocket, thus allowing more promiscuous binding [60**]. An alternative explanation is that the lectin domain contains additional weak carbohydrate-binding sites and mutations in these regions fortuitously change these weak binding sites to strong ones. Some plant lectins are known to contain multiple carbohydrate-binding sites — for example, the garlic lectin dimer contains seven mannose-binding sites [62]. A more complete understanding of the molecular details of FimH–carbohydrate interactions, of the structural basis of the differential binding of monomannose and oligomannose moieties, and of the structural basis of FimH-mediated bacterial invasion awaits further data.

**Conclusions: a molecular model for organelle biogenesis**

The binding of the pilus adhesin to its sugar receptor represents a critical early step in the interaction between a pathogen and its host. The studies of pili reviewed here have begun to elucidate the molecular mechanisms of bacterial attachment and the pathogenic consequences that follow. They also promise to shed light on general mechanisms of organelle biogenesis. For example, donor strand exchange produces a very stable interaction — subunit oligomers resist dissociation by SDS at room temperature — allowing the construction of a very large, sturdy organelle [48]. The contribution by one subunit of an element of the fold, be it a strand, helix or other component, to its neighboring subunit may represent one general mechanism by which cells construct large organelles [49]. Such an assembly mechanism would most probably require coordination and regulation by the cell. Recent work has identified a growing number of chaperones involved in organelle biogenesis in Gram-negative bacteria. For example, the flagellar axial proteins each contain an amphipathic helix at their C terminus. FlgN, a flagellar assembly protein, binds to the flagellar-hook-associated proteins FlgK and FlgL, via these C-terminal amphipathic helices and enhances their export [63**]. FljT, another
protein associated with flagellar assembly, binds to the flagellar filament cap protein FlID in a similar manner. Thus, it was proposed that FlgN and FltI act as chaperones for specific flagellar subunits by binding to their helical domains and preventing premature subunit oligomerization [63**]. Other work revealed that the export of the hook-type proteins FlgD and FlgE required FltI, suggesting that FltI may also act as a chaperone [64**]. Chaperones are also thought to play roles in the assembly of the type III secretion apparatus, which shares homology with the flagellum, and of the CSI pilus family members (assembled by the alternative chaperone-usher pathway) [8,65,66**,67**]. By analogy to both the PapD superfamily and the flagellar chaperones, one general function of these molecules may be to cap the strongly interactive surfaces of subunits until they reach the proper assembly site [49,63**,68], thus ensuring that organelle biogenesis proceeds smoothly and appropriately.

**Update**

Recent work has demonstrated that cavocae, membrane structures involved in macromolecular import and transmembrane signaling, play a role in bacterial entry into bone-marrow-derived mast cells (BMMCs) [69**]. CD48, a receptor for FimH-expressing *E. coli* [57], co-localized with cavocae in BMMCs. Cavocae disrupting and usurping agents inhibited bacterial entry into mast cells and cavocae-specific markers were recruited to sites of bacterial entry. Finally, intracellular bacteria co-fractionated with cavocae. It is proposed that this cavocae-mediated entry pathway allows FimH-expressing *E. coli* to bypass the normal phagocytic pathway and remain viable inside mast cells.

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**References and recommended reading**

Papers of particular interest, published within the annual period of review, have been highlighted as:

* of special interest
** of outstanding interest


39. The PapD chaperone can form a dimer, capping its own subunit-interacting surface. The authors present crystal structures of two PapD dimers.


45. The NMR structure of the FimC chaperone, which directs type 1 pilus assembly, reveals that it shares the same overall fold as PapD. Subtle differences between the two structures are identified.


48. The mapping of the surfaces of the FimC chaperone that interact with FimH in the soluble chaperone–adhesin complex. The interacting surfaces are essentially limited to the N-terminal domain, but include regions beyond those in the G3 strand that were already known.


50. This paper, together with [47]**, reveals the donor strand complementation mechanism of chaperone–subunit interaction. In these two crystal structures, the chaperone donates its G3 strand to complete the Ig fold of the subunit, simultaneously stabilizing it and capping its interactive surface. The two papers present a donor strand exchange model of pilus biogenesis in which the N-terminal extension of one subunit displaces the chaperone G3 strand during assembly and completes the Ig fold of its neighbor in the mature organelle. In the FimC–FimH structure, a putative mannoside-binding pocket was identified in the receptor-binding domain of FimH.


52. See annotation to [46]**.


57. Naturally occurring FimH variants bind mannosamine with either high or low affinity. These variants are found predominantly in uropathogenic and commensal strains, respectively. The authors present evidence that high-affinity mannose binding facilitates colonization of the urinary tract, whereas low-affinity mannose binding facilitates colonization of the oral cavity. Thus, it is proposed that FimH polymorphism arises by a selection mechanism that balances these two advantages and, hence, that genetic variation in an originally commensal trait can produce a virulence factor.


59. The authors present high-resolution electron microscopy images of the intimate attachment of uropathogenic E. coli to bladder epithelial cells mediated by type 1 pilus. The pilus makes contact with the underlying protein coat that coats the luminal surface of these cells. Infection by uropathogenic E. coli leads to the death and exfoliation of the epithelial cells by an apoptotic-like mechanism; however, some bacteria circumvent this innate host defense mechanism by invading the now exposed underlying cells.
Schembri MA, Sokurenko EV, Klemm P: both initial attachment and movement along the surface during this process.


Flagellar axial proteins, including hook-associated proteins (HAPs), are predicted to have amphipathic helices at their C termini. The cytoplasmic FimN protein specifically binds the HAPs FlgK and FlgL by their C-terminal helices and facilitates efficient flagellum assembly. FltB binds the filament cap protein FlgD by its C-terminal helix. It is proposed that FlgN and FltD are specific chaperones that prevent premature oligomerization of HAPs during flagellum biogenesis.


Eight general components – FljA, FliB, FliH, Flj, Flf, Flo, Flp, FlQ and Flr – of the flagellar export apparatus are identified. The authors demonstrate that Flf is required for the export of some substrates and that Flj is required for rod assembly. The cytoplasmic protein Flj is required for the export of FlgD and FlgE. It is proposed that Flj acts as chaperone for FlgD and FlgE during flagellum assembly.


YopN is required to prevent Yop secretion in Yersinia in the presence of calcium and before contact with the host cell. This paper presents evidence that YscB acts a specific chaperone for YopN.

Neyt C, Cornelis GR: Role of SycD, the chaperone of the Yersinia Yop translocators YopB and YopD. Mol Microbiol 1999, 31:143-156.

The authors demonstrate that SycD binds the putative pore-forming protein YopB, as well as YopB and YopD together. It was previously known that SycD binds the YopD translocator. The SycD-binding site on YopB could not be localized to a discrete linear polypeptide segment. It is proposed that SycD acts as a chaperone to prevent premature association of the YopB–YopD complex with LcrV.


The authors present evidence that FimH-expressing E. coli gain entry into mast cells by a distinct, caveolae-mediated pathway. They suggest that the bacteria use this pathway to elude the normal phagocytic killing mechanism of mast cells and remain viable inside these cells.