Multiple pathways allow protein secretion across the bacterial outer membrane

David G Thanassi* and Scott J Hultgren†

Secretion of proteins across the bacterial outer membrane takes place via a variety of mechanisms from simple one-component systems to complex multicomponent pathways. Secretion pathways can be organized into evolutionarily and functionally related groups, which highlight their relationship with organelle biogenesis. Recent work is beginning to reveal the structure and function of various secretion components and the molecular mechanisms of secretion.

Addresses
*Department of Molecular Genetics and Microbiology and Center for Infectious Diseases, 130 Life Sciences Building, State University of New York at Stony Brook, Stony Brook, New York 11794-5222, USA; e-mail: dthanassi@ms.cc.sunysb.edu
†Department of Molecular Microbiology, 660 South Euclid Avenue, Campus Box 8230, Washington University School of Medicine, St. Louis, Missouri 63110, USA; e-mail: hultgren@borcim.wustl.edu

Current Opinion in Cell Biology 2000, **12**:420–430

0955-0674/00/$ – see front matter

Abbreviations

ABC	ATP-binding cassette
GSP	general secretory pathway
IM	inner membrane
MFP	membrane fusion protein
OM	outer membrane
PT	pertussis toxin

Introduction

Protein secretion is required for numerous aspects of the bacterial life cycle, including organelle biogenesis, nutrient acquisition, and virulence-factor expression. Gram-negative bacteria face a special challenge in this regard, as secreted proteins must cross the periplasm and the outer membrane (OM) in addition to the cytoplasmic or inner membrane (IM). Secretion is defined in this review as any process in which a protein crosses the OM barrier. Thus, in addition to export of proteins to the extracellular medium, secretion includes export of proteins that remain anchored to the cell surface or are assembled into cell-surface organelles.

Secretion across the OM represents a different problem from export of unfolded proteins across the IM. Proteins acquire structure in the periplasm, including formation of disulfide bonds, and may completely fold before crossing the OM. In addition, ATP or other sources of energy are not known to be present at the OM. Export systems must therefore be self-energized or have mechanisms for harnessing the energy available at the IM. Gram-negative bacteria have evolved a number of pathways for protein secretion that can be organized into six overarching groups. Four of these pathways represent terminal branches of the general secretory pathway (GSP), exporting proteins with cleavable amino-terminal signal sequences that require the Sec system for translocation across the IM (see [1] for a recent Sec review). The other pathways are Sec-independent and capable of exporting substrates directly from the cytoplasm outside the bacteria. At least three pathways share secretion mechanisms with, and probably evolved from, organelle biogenesis systems, and one pathway is dedicated to the assembly of surface structures. This review will present a brief overview of each secretion pathway, focusing on secretion mechanisms and will highlight important advances over the past year. Readers are referred to recent reviews for in-depth discussions of each pathway [2–8].

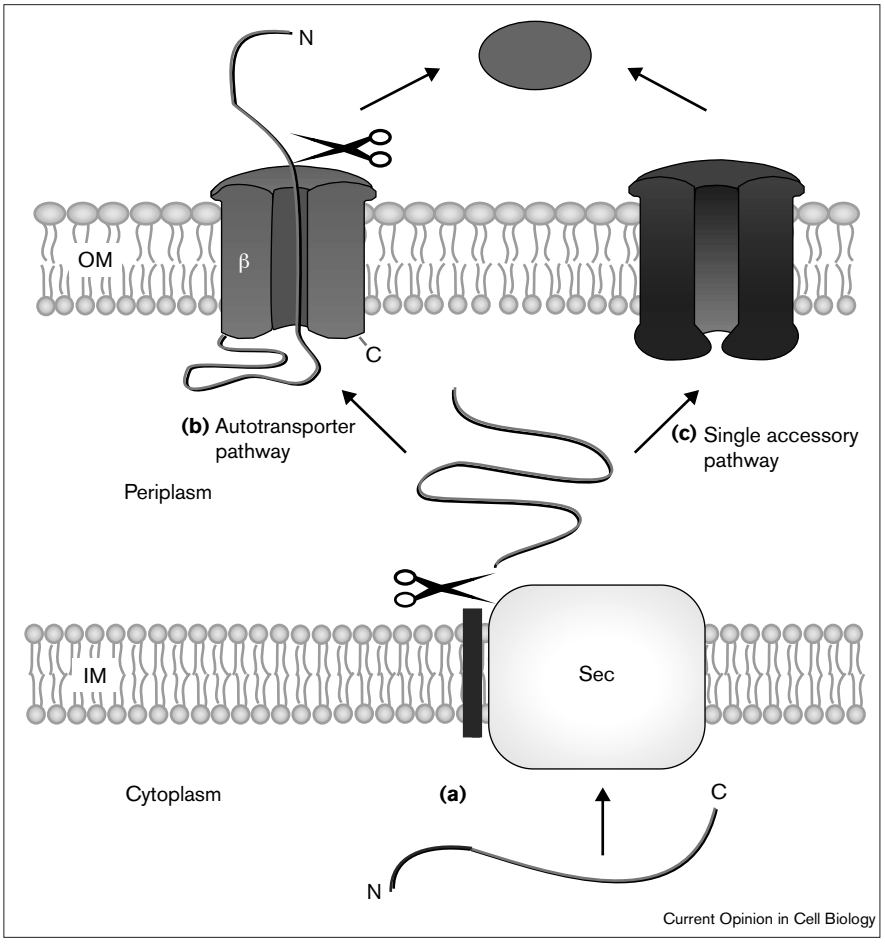
Autotransporters and proteins requiring single accessory factors

The autotransporter secretion pathway is a terminal branch of the GSP that exports proteins with diverse functionalities, including proteases, toxins, adhesins and invasins [2]. The prototypical member of the autotransporter family is the *Neisseria gonorrhoeae* IgA1 protease [9]. This pathway has also been termed type IV secretion. However, the type IV nomenclature has also been claimed for the much more complex DNA- and protein-exporting system described below. To avoid confusion, this review will use the more descriptive autotransporter designation.

Autotransporters do not require accessory factors for transit from the periplasm to the bacterial surface (Figure 1). A typical autotransporter contains three domains: an amino-terminal signal sequence for secretion across the IM by the Sec system, an internal passenger or functional domain, and a carboxy-terminal β-domain [2]. The β-domain inserts into the OM to form what is predicted to be a β-barrel pore structure, similar to the bacterial porins, through which the passenger domain passes to the cell surface. A linker region connecting the passenger and β-domains is also essential for export and may guide the passenger region through the β-domain channel [10]. Secretion across the OM does not appear to require input of external energy. Once secreted, the passenger domain is either retained on the bacterial surface or released into the environment by proteolysis. The nature of the passenger domain does not appear to be important, as foreign proteins can be substituted for this domain and successfully secreted [11,12].

Work on secretion of foreign proteins indicates that formation of disulfide bonds by the passenger domain hinders export to the cell surface, pointing to a requirement for unfolded or partially folded substrates [11,12]. However, this is contradicted by recent research showing export of a functional, disulfide-bonded antibody fragment fused to the IgA β-domain [13•]. Much attention has been focused on the structure of the β-domain. Computer modeling and gel-mobility studies support the formation

Figure 1



Models for the autotransporter and single accessory pathways. **(a)** Both pathways are branches of the GSP, and proteins cross the IM via the Sec system, followed by cleavage of their amino-terminal signal sequence in the periplasm by signal peptidase. **(b)** For autotransporters, the carboxy-terminal or β domain of the protein inserts into the OM to form a β-barrel secretion channel through which the passenger domain passes to the cell surface. The passenger domain is released into the extracellular medium by proteolysis. **(c)** In pathways requiring a single accessory factor, a separate protein forms the β-barrel OM channel, which may be gated.

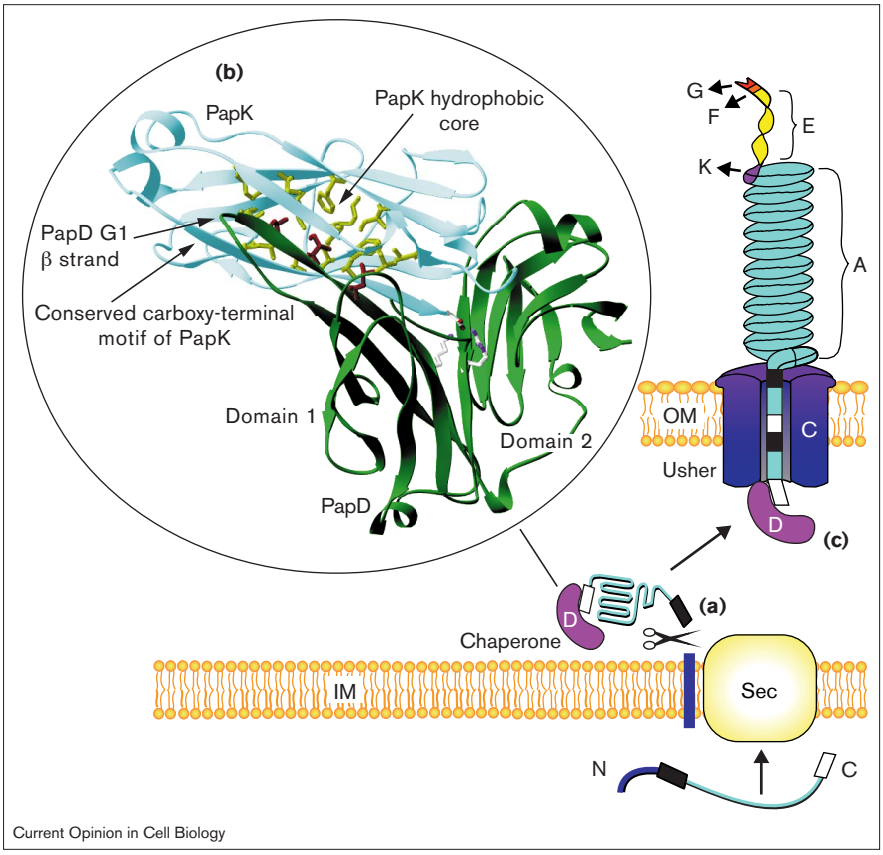
of a β-barrel structure [2,10]. The β-domain of the *Bordetella pertussis* autotransporter BrkA was recently shown to form pores in experimental lipid bilayers [14]. It is unknown whether a single β-domain forms the channel or if oligomerization is required.

A separate pathway has been identified that requires a single accessory factor for secretion across the OM. Examples include export of *B. pertussis* filamentous hemagglutinin and *Serratia marcescens* hemolysin by FhaC and ShlB, respectively [15,16]. The accessory protein is thought to form a β-barrel pore in the OM for secretion of the substrate to the cell surface (Figure 1). Foreign proteins can be secreted by this pathway, but acquisition of disulfide bonds appears to block export to the cell surface [17]. Recent work demonstrated that both FhaC and ShlB form channels in experimental lipid bilayers [15,16]. The channels may be gated and evidence suggests that ShlB acts as a monomer. This single accessory pathway appears to be functionally similar to the autotransporters, except that separate proteins encode the passenger and β-domains. However, there is no sequence homology between the two systems and the accessory factors appear instead to be related to a group of OM proteins that share homology with a protein-translocating porin found in the outer envelope of chloroplasts [18]. Thus, the autotransporter and single accessory pathways probably evolved independently and may, in fact, employ completely different mechanisms.

Chaperone/usher pathway

The chaperone/usher pathway is a branch of the GSP dedicated to the assembly and secretion of a broad range of adhesive virulence structures on the Gram-negative bacterial surface [3]. Secretion across the OM by this pathway requires only two components: a periplasmic chaperone and an OM protein termed an usher (Figure 2). The prototypical organelles assembled by this pathway are P and type 1 pili, expressed by uropathogenic *Escherichia coli* [19,20]. These pili are composite structures consisting of a thin, flexible tip fibrillum connected to a rigid, helical rod (Figure 2) [21]. Following export across the IM via the Sec pathway, pilus subunits interact with the periplasmic chaperone via a conserved carboxy-terminal motif present on each of the pilus subunits. The chaperone facilitates release of pilus subunits into the periplasm, guides their proper folding, and caps subunit-interactive surfaces to

Figure 2



Model for biogenesis of P pili by the chaperone/usher pathway. **(a)** Pilus subunits cross the IM via the Sec system, followed by cleavage of their amino-terminal signal sequence. The periplasmic chaperone PapD binds to each subunit via a conserved carboxy-terminal subunit motif (white box), allowing proper subunit folding and preventing premature subunit–subunit interactions. **(b)** The crystal structure of the PapD–PapK chaperone–subunit complex. The chaperone (green) consists of two Ig folds. The subunit (blue) consists of a single Ig fold that lacks the usual seventh β strand, resulting in exposure of its hydrophobic core. The G1 β strand of PapD binds to the conserved carboxy-terminal motif of PapK, donating its hydrophobic residues to complete the structure of the subunit in a mechanism termed donor strand complementation. **(c)** Chaperone–subunit complexes are targeted to the OM usher for assembly into pili and secretion across the OM. Subunit–subunit interactions are thought to take place by interaction of conserved amino-terminal (black box) and carboxy-terminal (white box) motifs. The amino-terminal motif of one subunit may complete the structure of the preceding subunit in a mechanism termed donor-strand exchange to build the pilus fiber. The usher channel is only able to allow passage of a linear fiber of folded subunits, forcing the pilus rod to adopt its final helical conformation at the cell surface. The location of Pap subunits in the pilus is indicated.

prevent premature interactions in the periplasm [22]. Next, periplasmic chaperone–subunit complexes are targeted to the OM usher [23,24].

Interaction with the usher triggers chaperone dissociation from the subunit by an unknown mechanism, exposing subunit-assembly surfaces and allowing incorporation of the subunit into the pilus fiber. The usher provides a translocation channel through the OM for secretion of the pilus. The P pilus usher, PapC, has been imaged by electron microscopy [3] and revealed as ring-shaped oligomeric structures containing central pores 2–3 nm in diameter. Pore formation was confirmed by reconstitution of purified usher protein into liposomes [3]. Interestingly, OM components of the unrelated type II and type III secretion systems also form ring-shaped oligomeric complexes with central pores (see below). A 2–3 nm usher channel would be sufficient to allow passage of folded subunits assembled into a linear fiber, such as the pilus tip fibrillum, but not the helical pilus rod. Therefore, the pilus rod would be constrained to a linear fiber of folded subunits while traversing through the usher. It would only be able to coil into its final helical conformation once it reached the cell surface (Figure 2). The chaperone/usher pathway does not appear to require input of external energy for secretion of pili across the OM [25] and coiling of the rod outside the cell may facilitate the outward translocation of pili.

Recently, crystal structures of FimC–FimH and PapD–PapK (corresponding to chaperone-subunit complexes from type 1 and P pili, respectively) have been solved (Figure 2) [26••,27••]. The chaperone consists of two immunoglobulin-like (Ig) domains oriented in an L-shape. The FimH adhesin also consists of two domains: a receptor-binding domain and a pilin domain. The pilin domain of FimH and the single domain of the PapK subunit have Ig folds that lack the seventh (carboxy-terminal) β-strand present in canonical Ig folds. This results in a deep groove along the surface of the subunit and exposure of its hydrophobic core.

In the chaperone–subunit complexes, the chaperone donates its G1 β-strand and a portion of its F1-G1 loop to complete the Ig fold of the subunit by occupying the groove (Figure 2). This donor-strand complementation interaction stabilizes the subunit by shielding its hydrophobic core. Indeed, alternating hydrophobic residues of the G1 strand become an integral part of the hydrophobic core of the subunit. Part of the groove is formed by the conserved carboxy-terminal subunit motif, which has also been

implicated in subunit–subunit interactions [28]. Thus, the donor-strand complementation interaction caps an interactive surface and prevents premature pilus formation in the periplasm. Indeed, the folding, stabilization, and capping of the subunit may be part of a single dynamic process that ensures subunit interactive surfaces are not accessible until pilus biogenesis at the usher.

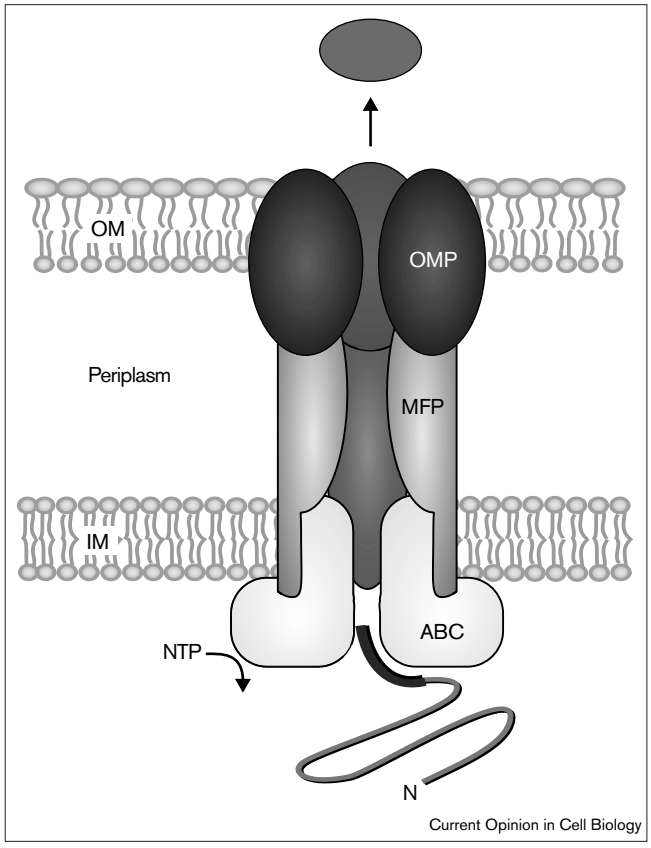
The crystal structures suggest a model for pilus assembly. Subunits also have an amino-terminal extension with a conserved alternating hydrophobic motif that participates in subunit–subunit interactions [28]. This motif is similar to the alternating hydrophobic motif present in the chaperone G1 β-strand. The amino-terminal extension projects away from the subunit, where it would be free to interact with another subunit [27••]. During pilus biogenesis, the amino-terminal extension of one subunit may displace the chaperone G1 β-strand from its neighboring subunit in a mechanism termed donor-strand exchange. The mature pilus would thus consist of an array of Ig domains, each of which contributes a strand to the fold of the preceding subunit to produce a highly stable organelle.

Type I secretion

Type I or ATP-binding cassette (ABC) protein exporters are employed by a wide range of Gram-negative bacteria for secretion of toxins, proteases and lipases [4]. Secretion of α-hemolysin by *E. coli* represents the prototypical type I exporter [29]. Related ABC systems are conserved from prokaryotes to eukaryotes and export a variety of toxic compounds and antibiotics. The type I pathway is Sec independent and secretes proteins directly from the cytoplasm across the OM without a periplasmic intermediate. Substrates of this pathway lack a cleavable amino-terminal signal sequence, instead, they possess a carboxy-terminal ~60 amino acid secretion signal [4]. The type I export apparatus consists of three proteins: an IM ABC exporter, an IM-anchored protein that spans the periplasm, termed a membrane fusion protein (MFP), and an OM protein (OMP) (Figure 3).

TolC, the OMP for hemolysin export, assembles as a trimeric complex in the OM and is predicted to consist of a porin-like β-barrel membrane domain with a carboxy-terminal hydrophilic region that extends into the periplasm [30]. However, recent sequence analysis indicates that TolC and other OMPs are unrelated to porins [31•]. The OMP presumably functions as the OM secretion channel. Indeed, pore-forming activity by TolC oligomers has been demonstrated in experimental lipid bilayers [32]. The periplasmic MFP component also assembles as a trimer and interacts with both the OMP and ABC exporter [29,33]. MFPs typically contain a hydrophobic amino terminus believed to span the IM or are anchored in the IM by lipid modification of the amino terminus [31•]. The bulk of the MFP is thought to extend across the periplasm to contact the OMP and/or the OM itself. The MFP is thought to facilitate substrate secretion

Figure 3



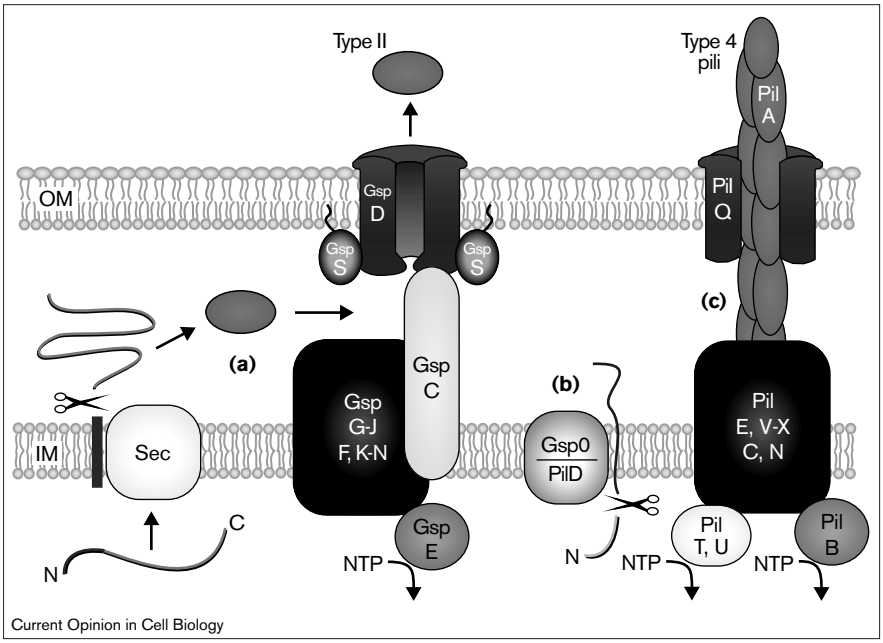
Model for secretion by the type I pathway. Type I substrates do not posses an amino-terminal signal sequence recognized by the Sec system, instead they possess a carboxy-terminal signal sequence that is not cleaved. During secretion, the periplasmic MFP interacts with both the IM ABC exporter and the OM channel-forming protein (OMP) to allow secretion to the extracellular medium without a periplasmic intermediate. Both the OMP and MFP are thought to assemble as trimers. ATPase activity by the ABC protein may energize substrate release into the medium.

without a periplasmic intermediate by forming a closed bridge or channel across the periplasm or by fusing the inner and outer membranes, allowing direct contact of the ABC exporter and OMP channel.

Experimental evidence has led to two related models for secretion by the type I pathway. Work on *E. coli* hemolysin secretion [29] indicates that the ABC exporter and MFP associate before substrate binding. Substrate binding to this complex then triggers contact of the MFP with the OMP. This bridging is transient, collapsing after export of the substrate. ATP hydrolysis by the ABC exporter drives substrate release outside the cell and is not required for substrate binding or assembly of the complex.

Work on *S. Marcescens* hemoprotein and *Erwinia chrysanthemi* metalloprotease secretion points towards a slightly different order of events [33]. In this model, the ABC exporter and MFP do not associate before substrate

Figure 4



Models for type II secretion and type 4 pilus biogenesis. Components of the type II secreton are indicated using the Gsp nomenclature, and type 4 pilus proteins are labeled according to the *Pseudomonas aeruginosa* Pil system. Similar shading and location indicates homologous components. **(a)** Type II substrates cross the IM via the Sec system followed by signal-sequence cleavage and protein folding in the periplasm. The GspD secretin, indicated here as a complex with the GspS lipoprotein, serves as a gated channel for secretion of substrates to the cell surface. GspC may transmit energy from the IM, presumably generated by the cytoplasmic GspE nucleotide-binding protein, to the OM complex. GspG–J exhibit homology to the pilin subunit PilA and are processed by the GspO prepilin peptidase. **(b)** GspO cleaves their amino-terminal leader sequence on the cytoplasmic face of the IM. In *P. aeruginosa* the type 4 prepilin peptidase PilD is the same protein as GspO. **(c)** Type 4 pilus biogenesis requires the OM secretin PilQ. The type 4 pilus system contains at least four pilin-like components (PilE and PilV–X), in addition to PilA, that are processed by PilD. Additional nucleotide-binding proteins (PilT, U) are present and are involved in a pilus-generated movement termed 'twitching motility'.

binding. Instead, an ordered set of associations takes place in which the substrate first binds to the ABC exporter, which then triggers binding of the MFP. This complex in turn interacts with the OMP, allowing secretion of the substrate. Future work is needed to resolve these two models or to determine if they reflect a variation among individual type I mechanisms.

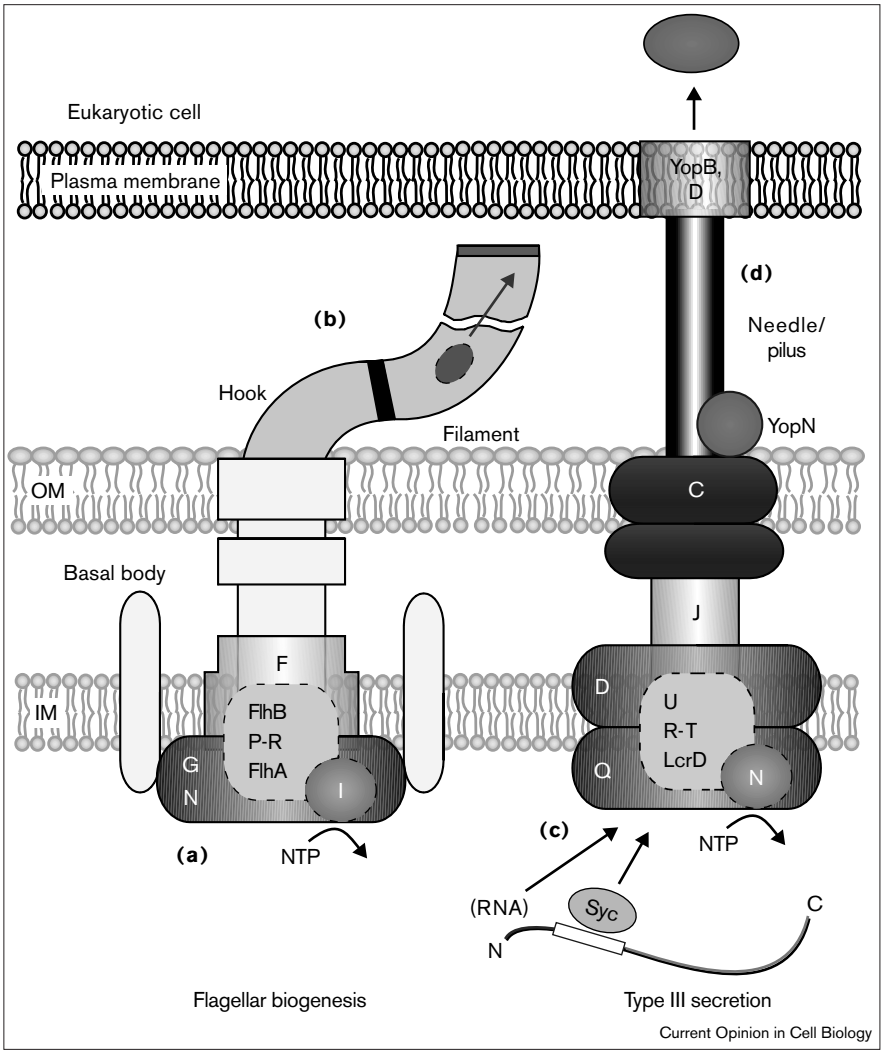
Type II secretion

The type II secretory pathway represents a third terminal branch of the GSP, also referred to as the main terminal branch. This pathway is responsible for secretion of extracellular enzymes and toxins by a wide variety of Gram-negative bacteria [6,34,35]. Pullulanase (*pul*) secretion by *Klebsiella oxytoca* represents the prototypical type II pathway [35]. The type II secretory pathway is closely related to biogenesis of type 4 pili — long, polarly localized pili important for the virulence of many bacterial pathogens [5]. It should be clarified that type II does not refer to the GSP in general, but is one of several terminal branches as described in this review.

Secretion across the OM by the type II branch is much more complex than the autotransporter and chaperone/usher branches, requiring between 12 and 16 accessory proteins, collectively referred to as the secreton (Figure 4) [35]. A unified type II nomenclature has been established, using the designation Gsp (perhaps reinforcing confusion of type II secretion with the GSP) and giving each homologous secreton component a letter based on the *pul* system. The type II secreton includes two OM components: GspD and GspS [34,35]. GspD is an integral OM protein and GspS is a small lipoprotein required in at least some type II systems for proper targeting and insertion of GspD in the OM [36•]. GspD belongs to the secretin superfamily, members of which are also required for type 4 pilus biogenesis, filamentous phage biogenesis, and type III secretion (see below) [37]. Secretins form highly stable ring-shaped complexes of 12–14 subunits with central channels ranging from 5–10 nm in diameter, large enough to accommodate folded substrates [38–41]. Secretins share structural similarity with the ring-shaped usher proteins, but there is no sequence homology between these two families. Whether they utilize similar secretion mechanisms remains to be determined.

The PulD secretin was recently imaged by cryo-electron microscopy as a complex with the PulS lipoprotein [36•]. Three-dimensional reconstruction revealed a 7.5 nm central channel formed by two stacked rings, with a set of radial spokes, presumably formed by PulS, emanating from one ring. The PulDS complex, as well as isolated secretins from *Pseudomonas* and f1 phage, form channels in experimental lipid bilayers and there is strong evidence for channel gating [36•,42•,43]. The amino-terminal half of secretins may serve as the channel gate, whereas the carboxyl terminus appears to direct oligomerization and contain the channel-forming activity [43,44].

Figure 5



Models for type III secretion and flagellar biogenesis. The type III system is depicted on the right and flagellar biogenesis on the left. Similar shading and location indicates homologous components. Components of the type III apparatus are labeled according to the *Yersinia* nomenclature (Ysc proteins or as indicated). Labels on the flagellar apparatus correspond to the Fli proteins, except as indicated. Only components of the flagellar basal body sharing homology with the type III system are shown. **(a)** Flagellar basal body components FlhA–B and FliP–R may form a central secretion apparatus for export of flagellin and other proteins, energized by the FliI ATPase. **(b)** Flagellin subunits travel through a central channel in the basal body, then the hook and filament, before incorporation at the distal end of the filament. **(c)** Some type III secretion substrates contain two amino-terminal signal sequences for targeting to the secretion machinery, one encoded by the mRNA and the second serving as a binding site for cytoplasmic Syc chaperones. By homology with flagellar proteins, YscR–U and LcrD may form a central secretion apparatus, energized by YscN. The YscC secretin presumably provides a secretion channel across the OM and the surface-localized YopN protein is thought to serve as a channel gate. **(d)** By analogy with flagella, type III substrates (Yop proteins) may travel through a central channel in the type III needle or pilus. Translocation of Yops into the target eukaryotic cell may take place via a channel formed in the plasma membrane by YopB and YopD.

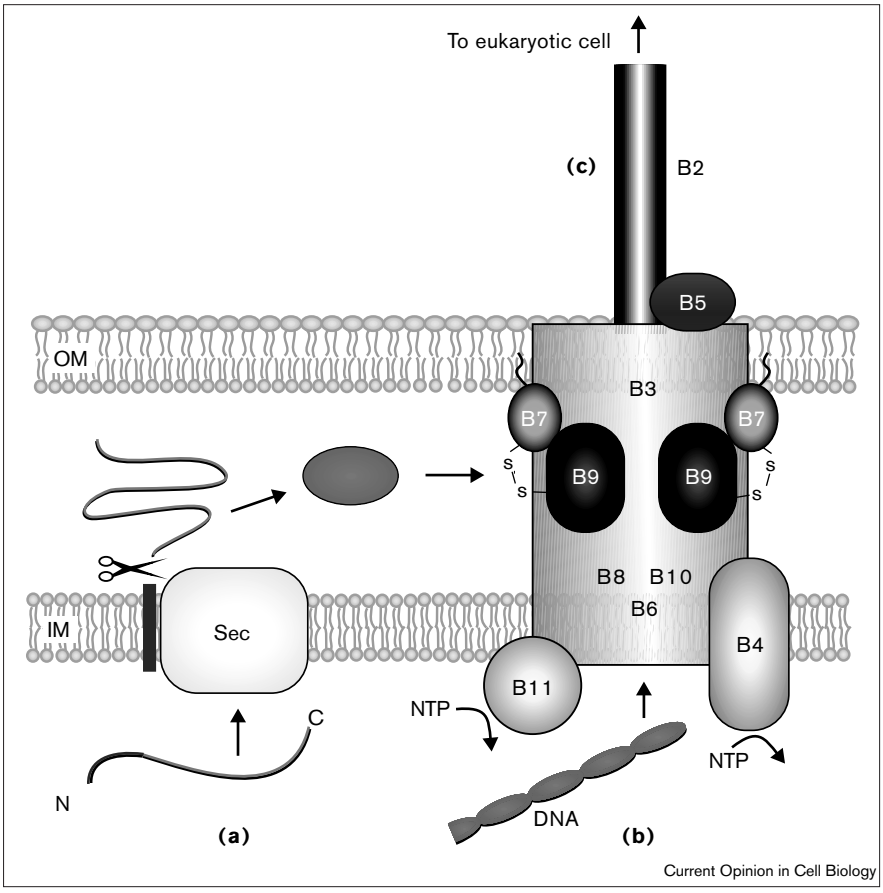
Surprisingly, most secreton components are associated with the IM (Figure 4) [34]. At least four IM proteins, GspG, H, I, and J, exhibit limited homology to the type 4 pilus structural subunit, pilin. These 'pseudopilins' undergo processing by the GspO IM protein, a prepilin peptidase interchangeable with the type 4 pilus prepilin peptidase (Figure 4) [45]. The pseudopilins have been proposed to assemble into a pilus-like structure that spans the periplasm, although evidence for such a structure has not been found [46,47].

Secreton components GspC, F, K, L, M and N are also IM proteins, all with extensive periplasmic domains. GspE is a cytoplasmic protein that localizes to the IM via interaction with GspL [48,49]. GspE contains a conserved ATP-binding motif and has autokinase activity [48,50]. GspE may regulate secretion or energize the secretion process and/or assembly of the secreton. Accumulated data points towards extensive protein–protein interactions among the IM components [47,49,51]. This leads to a model in which GspE uses ATP to effect conformational changes in the IM proteins that are transmitted to the periplasmic domains and then to the OM. GspC, which fractionates with both the IM and OM, may be responsible for energy transduction to GspD in the OM in a manner similar to that proposed for the role of TonB in siderophore-mediated iron import [52,53].

Type III secretion

Type III secretion pathways capable of translocating anti-host factors into the cytosol of target eukaryotic cells have been identified in a number of animal and plant pathogens [7]. Secretion of *Yersinia* outer proteins (Yops) by *Yersinia* spp. represents the prototypical type III export pathway. Translocation of Yops into the cytosol of target cells disrupts signaling pathways, allowing the bacteria to evade host defense mechanisms [54]. Type III secretion is Sec independent, may take place without a periplasmic intermediate, and requires ~20 secretion components that assemble into a large structure that spans both bacterial membranes and possibly the host cell membrane as well

Figure 6



Model for type IV secretion. Components of the type IV apparatus are labeled according to the VirB system. **(a)** Protein secretion by the type IV pathway may take place via a periplasmic intermediate, with substrates first traversing the IM via the Sec pathway. **(b)** DNA secretion probably takes place from the cytoplasm in a single step, without a periplasmic intermediate. VirB4 and B11 contain nucleotide-binding activity and probably energize aspects of the secretion process. The lipoprotein VirB7 forms a disulfide-linked complex with VirB9 that may nucleate assembly of the type IV apparatus at the OM. **(c)** VirB2 is the major type IV pilus component and VirB5 is a minor pilus component. The pilus may serve as a secretion tube for translocation of proteins or DNA into target eukaryotic cells.

(Figure 5). Type III secretion shares one homologous component with the type II pathway: secretion across the OM requires a member of the secretin family of channel-forming proteins. The *Yersinia* and *Salmonella typhimurium* secretins YscC and InvG, respectively, have been imaged as ring-shaped complexes with large central pores [39,40]. Proper targeting of InvG to the OM requires the InvH lipoprotein, analogous to the role of GspS in the type II pathway.

The majority of type III components are thought to localize to the IM and are closely related to components of the flagellar basal body (Figure 5) [7]. The flagellar basal body spans the inner and outer membranes and provides an anchor for the flagellar filament [55]. The cytoplasmic face of the basal body is thought to contain the machinery that drives secretion and assembly of flagella via ATP hydrolysis. This machinery exports flagellin monomers through a central channel within the basal body and filament for assembly at the distal end of the growing flagellum. Interestingly, the flagellar apparatus was recently shown to function as a protein secretion system [56].

The *S. typhimurium* and *Shigella flexneri* type III secretion structures have recently been visualized by electron microscopy [57,58••]. The *S. typhimurium* structure is strikingly similar to the flagellar basal body, with a hollow projection extending out from the bacterial surface, termed the needle, in place of a flagellar filament. In addition, the *S. flexneri* structure revealed a large bulb region that presumably extends into the bacterial cytoplasm. This bulb may represent the intact IM machinery that drives assembly and secretion in both the type III and flagellar systems. Both plant and animal type III systems assemble long pili [59,60], possibly through extension of the needle structure. By analogy with flagella, this type III pilus may serve as a secretion tube allowing protein transport within its length.

Type III secretion is highly regulated and two amino-terminal secretion signals have been identified for export of Yop proteins (Figure 5). The first signal appears to reside in the mRNA and may target the RNA–ribosome complex to the type III machinery for coupled translation and secretion, as recently demonstrated for YopQ [61•]. The second Yop secretion signal serves as the binding site for cytoplasmic chaperones termed Syc proteins and may specifically target Yops to the type III machinery for translocation into host cells [62].

Of great interest is the mechanism of translocation across the host cell membrane. Most models postulate pore

formation in the target cell by type-III-secreted proteins. The *Yersinia* YopB and YopD proteins are good candidates for such an activity [63••,64]. YopB and YopD are substrates of the secretion apparatus, yet are required for translocation of Yop effector proteins into host cells. *Yersinia* and *S. flexneri* expressing type III systems create pores in red blood cell membranes, causing hemolysis [58••,65]. Close contact is required for lysis, which is dependent on YopB in *Yersinia* and the YopB and YopD homologs, IpaB and IpaC, in *S. flexneri*. YopB and YopD are both required for pore formation in macrophage membranes, and both proteins were inserted by the type III system into liposomes [63••,64]. Fusion of these liposomes with experimental lipid bilayers resulted in channel formation, which was dependent on YopB and modified by YopD [63••]. Thus, YopB and YopD may be inserted by the type III secretion apparatus into the plasma membrane of target cells to form a pore for translocation of effector Yops into the host cytosol (Figure 5). However, a recent report questions the role of YopB in translocation [66] and this model remains to be proven.

Type IV secretion

Type IV secretion is a recently identified protein secretion pathway homologous to the IncP and IncN bacterial conjugation systems and the VirB system of *Agrobacterium tumefaciens* that facilitates translocation of oncogenic T-DNA into plant cells [8]. Type IV homologs have been identified in *Legionella pneumophila* and *Helicobacter pylori*. A type IV system is also required for pertussis toxin (PT) secretion by *B. pertussis* [67-70]. PT export requires nine gene products of the *ptl* locus, all with homologs in the *virB* locus of *A. tumefaciens*, which contains 11 genes. DNA export by the VirB and conjugal systems is postulated to take place in a single step from the cytoplasm to the outside of the cell. However, evidence strongly suggests PT export occurs in two steps, with the toxin subunits first traversing the IM via the Sec system [8]. Thus, protein export by the type IV pathway may represent a fourth branch of the GSP.

Most information about the type IV secretory apparatus comes from studying the VirB system (Figure 6). A number of VirB proteins are membrane-associated, interact with each other and themselves, and are present in multiple copies. Proteins VirB7–10 fractionate with both the inner and outer membranes and are proposed to contain large periplasmic domains, suggesting the formation of a complex that spans the periplasm [71,72]. VirB7 is an OM-associated lipoprotein that forms a disulfide-bonded heterodimeric complex with VirB9 [73]. VirB7 and B9 are important for the stability of other VirB proteins, which leads to a model in which the B7–B9 complex at the OM nucleates and stabilizes assembly of the secretion apparatus [73]. There is no secretin homolog in the type IV system and the protein or proteins that form the OM secretion channel have yet to be defined. VirB4 and B11 localize to the IM and possess ATPase and/or autokinase activity [74,75]. Mutations in the consensus nucleotide-binding domain of VirB4 exert a dominant-negative effect on secretion, whereas VirB11 mutants are recessive [76]. However, both proteins are thought to act as multimers and to interact with transport components. A subset of VirB proteins consisting of VirB3,4 and B7–10 facilitates uptake of conjugal plasmid DNA [77] and may represent a core type IV transport complex. Interestingly, VirB4 oligomerization, but not nucleotide-binding activity, is required for function of this minimal complex [76]. Thus, protein–protein interactions may drive assembly of a minimal transport complex, and nucleotide-binding activity may be required to configure this complex for secretion.

Reminiscent of the type III pathway, the VirB system assembles pili. VirB2 constitutes the major pilus subunit [78]. VirB2 and the IncP conjugative pilus subunit TrbC are post-translationally processed to a cyclic form via intramolecular head-to-tail peptide bonds [79•]. This novel configuration may confer stability to the pilus, analogous to the donor-strand exchange mechanism described above for chaperone/usher pili. It is tempting to speculate that the VirB pilus mediates cell–cell contact and provides a channel for direct translocation of the T-DNA complex into the host. Whether type IV protein exporters, such as the *ptl* secretion system, assemble pili or mediate direct transfer of proteins into host cells, akin to injection by the type III pathway, remains to be determined.

Conclusions

A vast amount of information has been gathered about protein secretion in Gram-negative bacteria, spurred on by the realization of the importance of these pathways in bacterial pathogenesis. A wide range of secretion strategies has been uncovered, from simple one-component systems to complex multicomponent assemblies. Relationships with organelle biogenesis mechanisms have informed our understanding of a number of these pathways. However, much is still unknown about the structure of the various secretion machineries, their molecular mechanisms of action, and how the machineries themselves are assembled. Future work in this direction will answer important cell biological questions and will also present attractive targets for the development of antimicrobial therapeutics.

Update

The link between type II secretion and type 4 pilus assembly was demonstrated in a recent publication by Sauvonnet *et al*. [80]. They showed that expression of the pullulanase type II secretion system of *K. oxytoca* in *E. coli* resulted in assembly of the PulG 'pseudopilin' into pilus-like bundles on the bacterial surface. In addition, this system could assemble the *E. coli* type 4 pilin protein PpdD into pili.

Recent work by Odenbreit *et al*. [81] on the type IV secretion system of *H. pylori* demonstrated that this system is able to translocate the CagA protein into the cytosol of gastric epithelial cells where it modulates host cell proteins.

Acknowledgements

We would like to thank J Bliska and C Stathopoulos for critical review of the manuscript. SJH is supported by National Institutes of Health grants R01AI29549 and R01DK51406.

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

1. Economou A: **Following the leader: bacterial protein export through the Sec pathway.** *Trends Microbiol* 1999, **7**:315-320.

2. Henderson IR, Novarro-Garcia F, Nataro JP: **The great escape: structure and function of the autotransporter proteins.** *Trends Microbiol* 1998, **6**:370-378.

3. Thanassi DG, Saulino ET, Hultgren SJ: **The chaperone/usher pathway: a major terminal branch of the general secretory pathway.** *Curr Opin Microbiol* 1998, **1**:223-231.

4. Binet R, Létoffé S, Ghigo JM, Delepelaire P, Wandersman C: **Protein secretion by Gram-negative bacterial ABC exporters — a review.** *Gene* 1997, 192:7-11.

5. Nunn D: **Bacterial type II protein export and pilus biogenesis: more than just homologies?** *Trends Cell Biol* 1999, **9**:402-408.

6. Stathopoulos C, Hendrixson DR, Thanassi DG, Hultgren SJ, St. Geme III JW, Curtiss III R: **Secretion of virulence determinants by the general secretory pathway in Gram-negative pathogens: an evolving story.** *Microbes Infect* 2000, in press.

7. Hueck CJ: **Type III protein secretion systems in bacterial pathogens of animals and plants.** *Microbiol Molec Biol Rev* 1998, **62**:379-433.

8. Burns DL: **Biochemistry of type IV secretion.** *Curr Opin Microbiol* 1999, **2**:25-29.

9. Pohlner J, Halter R, Beyreuther K, Meyer TF: **Gene structure and extracellular secretion of *Neisseria gonorrhoeae* IgA protease.** *Nature* 1987, **325**:458-462.

10. Maurer J, Jose J, Meyer TF: **Characterization of the essential transport function of the AIDA-I autotransporter and evidence supporting structural predictions.** *J Bacteriol* 1999, **181**:7014-7020.

11. Suzuki T, Lett M-C, Sasakawa C: **Extracellular transport of VirG protein in *Shigella*.** *J Biol Chem* 1995, **270**:30874-30880.

12. Klauser T, Pohlner J, Meyer TF: **Extracellular transport of cholera toxin B subunit using *Neisseria* IgA protease β-domain: conformation-dependent outer membrane translocation.** *EMBO J* 1990, **9**:1991-1999.

13. • Veiga E, de Lorenzo V, Fernández A: **Probing secretion and translocation of a β-autotransporter using a reporter single-chain Fv as a cognate passenger domain.** *Mol Microbiol* 1999, **33**:1232-1243.

This paper analyzes secretion by the autotransporter pathway using a single-chain antibody fragment fused to the β-domain of IgA protease. The paper challenges the notion that only unfolded passenger domains can be secreted across the OM.

14. Shannon JL, Fernandez RC: **The C-terminal domain of the *Bordetella pertussis* autotransporter BrkA forms a pore in lipid bilayer membranes.** *J Bacteriol* 1999, **181**:5838-5842.

15. Könninger UW, Hobbie S, Benz R, Braun V: **The haemolysin-secreting ShlB protein of the outer membrane of *Serratia marcescens*: determination of surface-exposed residues and formation of ion-permeable pores by ShlB mutants in artificial lipid bilayer membranes.** *Mol Microbiol* 1999, **32**:1212-1225.

16. Jacob-Dubuisson F, El-Hamel C, Saint N, Guédin S, Willery E, Molle G, Locht C: **Channel formation by FhaC, the outer membrane protein involved in the secretion of the *Bordetella pertussis* filamentous hemagglutinin.** *J Biol Chem* 1999, **274**:37731-37735.

17. Guédin S, Willery E, Locht C, Jacob-Dubuisson F: **Evidence that a globular conformation is not compatible with FhaC-mediated secretion of the *Bordetella pertussis* filamentous haemagglutinin.** *Mol Microbiol* 1998, **29**:763-774.

18. Reumann S, Davila-Aponte J, Keegstra K: **The evolutionary origin of the protein-translocating channel of chloroplastic envelope membranes: identification of a cyanobacterial homolog.** *Proc Natl Acad Sci USA* 1999, **96**:784-789.

19. Roberts JA, Marklund B-I, Ilver D, Haslam D, Kaack MB, Baskin G, Louis M: **The Galα(1-4)Gal-specific tip adhesin of *Escherichia coli* P-fimbriae is needed for pyelonephritis to occur in the normal urinary tract.** *Proc Natl Acad Sci USA* 1994, **91**:11889-11893.

20. Langermann S, Palaszynski S, Barnhart M, Auguste G, Pinkner JS, Burlein J, Barren P: **Prevention of mucosal *Escherichia coli* infection by FimH-adhesin-based systemic vaccination.** *Science* 1997, **276**:607-611.

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

22. Jones CH, Danese PN, Pinkner JS, Silhavy TJ, Hultgren SJ: **The chaperone-assisted membrane release and folding pathway is sensed by two signal transduction systems.** *EMBO J* 1997, **16**:6394-6406.

23. Saulino ET, Thanassi DG, Pinkner JS, Hultgren SJ: **Ramifications of kinetic partitioning on usher-mediated pilus biogenesis.** *EMBO J* 1998, **17**:2177-2185.

24. Thanassi DG, Saulino ET, Lombardo M-J, Roth R, Heuser J, Hultgren SJ: **The PapC usher forms an oligomeric channel: implications for pilus biogenesis across the outer membrane.** *Proc Natl Acad Sci USA* 1998, **95**:3146-3151.

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

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

See annotation [27••].

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

This paper and [26••] present the first crystal structures of chaperone-subunit complexes from the chaperone/usher pathway. The paper reveals that the periplasmic chaperone employs a novel mechanism of action, termed donor strand complementation, to complete the fold of the subunit and suggests a model for subunit-subunit interactions in the pilus.

28. Soto GE, Dodson KW, Ogg D, Liu C, Heuser J, Knight S, Kihlberg J: **Periplasmic chaperone recognition motif of subunits mediates quaternary interactions in the pilus.** *EMBO J* 1998, **17**:6155-6167.

29. Thanabalu T, Koronakis E, Hughes C, Koronakis V: **Substrate-induced assembly of a contiguous channel for protein export from *E. coli*: reversible bridging of an inner-membrane translocase to an outer membrane exit pore.** *EMBO J* 1998, **17**:6487-6496.

30. Koronakis V, Li J, Koronakis E, Stauffer K: **Structure of TolC, the outer membrane component of the bacterial type I efflux system, derived from two-dimensional crystals.** *Mol Microbiol* 1997, **23**:617-626.

31. • Johnson JM, Church GM: **Alignment and structure prediction of divergent protein families: periplasmic and outer membrane proteins of bacterial efflux pumps.** *J Mol Biol* 1999, **287**:695-715.

This paper presents an interesting sequence analysis and structure prediction strategy, applied to the MFP and OMP components of the type I secretory pathway.

32. Benz R, Maier E, Gentschev I: **TolC of *Escherichia coli* functions as an outer membrane channel.** *Int J Med Microbiol Virol Parasitol Infect Dis* 1993, **278**:187-196.

33. Letoffe S, Delepelaire P, Wandersman C: **Protein secretion in gram-negative bacteria: assembly of the three components of ABC protein-mediated exporters is ordered and promoted by substrate binding.** *EMBO J* 1996, **15**:5804-5811.

34. Russel M: **Macromolecular assembly and secretion across the bacterial cell envelope: type II protein secretion systems.** *J Mol Biol* 1998, **279**:485-499.

35. Pugsley AP, Francetic O, Possot OM, Sauvonnet N, Hardie KR: **Recent progress and future directions in studies of the main**

terminal branch of the general secretory pathway in Gram-negative bacteria — a review. *Gene* 1997, **192**:13-19.

36. • Nouwen N, Ranson N, Saibil H, Wolpensinger B, Engel A, Ghazi A, Pugsley AP: **Secretin PulD: association with pilot PulS, structure, and ion-conducting channel formation.** *Proc Natl Acad Sci USA* 1999, **96**:8173-8177.
This paper describes the purification of the secretin PulD as a stable complex with the PulS lipoprotein and analysis of this complex by cryo-electron microscopy. A three-dimensional reconstruction of the complex is presented, together with evidence that the complex forms channels in lipid bilayers.

37. Genin S, Boucher CA: **A superfamily of proteins involved in different secretion pathways in gram-negative bacteria: modular structure and specificity of the N-terminal domain.** *Mol Gen Genet* 1994, **243**:112-118.

38. Bitter W, Koster M, Latijnhouwers M, de Cock H, Tommassen J: **Formation of oligomeric rings by XcpQ and PilQ, which are involved in protein transport across the outer membrane of *Pseudomonas aeruginosa*.** *Mol Microbiol* 1998, **27**:209-219.

39. Crago AM, Koronakis V: ***Salmonella* InvG forms a ring-like multimer that requires the InvH lipoprotein for outer membrane localization.** *Mol Microbiol* 1998, **30**:47-56.

40. Koster M, Bitter W, de Cock H, Allaoui A, Cornelis GR, Tommassen J: **The outer membrane component, YscC, of the Yop secretion machinery of *Yersinia enterocolitica* forms a ring-shaped multimeric complex.** *Mol Microbiol* 1997, **26**:789-797.

41. Linderoth NA, Simon MN, Russel M: **The filamentous phage pIV multimer visualized by scanning transmission electron microscopy.** *Science* 1997, **278**:1635-1638.

42. • Marciano DK, Russel M, Simon SM: **An aqueous channel for filamentous phage export.** *Science* 1999, **284**:1516-1519.
This paper presents lipid-bilayer analysis of the secretin pIV and a pIV mutant with altered permeability characteristics. The paper provides strong evidence that secretins contain large gated channels.

43. Brok R, Van Gelder P, Winterhalter M, Ziese U, Koster AJ, de Cock H, Koster M: **The C-terminal domain of the *Pseudomonas* Secretin XcpQ forms oligomeric rings with pore activity.** *J Mol Biol* 1999, **294**:1169-1179.

44. Guilvout I, Hardie K, Sauvonnet N, Pugsley AP: **Genetic dissection of the outer membrane secretin PulD: are there distinct domains for multimerization and secretion specificity?** *J Bacteriol* 1999, **181**:7212-7220.

45. Strom MS, Nunn D, Lory S: **Multiple roles of the pilus biogenesis protein, PilD: involvement of PilD inexcretion of enzymes from *Pseudomonas aeruginosa*.** *J Bacteriol* 1991, **173**:1175-1180.

46. Hobbs M, Mattick JS: **Common components in the assembly of type 4 fimbriae, DNA transfer systems, filamentous phage and protein-secretion apparatus: a general system for the formation of surface-associated protein complexes.** *Mol Microbiol* 1993, **10**:233-243.

47. Pugsley AP: **Multimers of the precursor of a type IV pilin-like component of the general secretory pathway are unrelated to pili.** *Mol Microbiol* 1996, **20**:1235-1245.

48. Sandkvist M, Bagdasarian M, Howard SP, DiRita VJ: **Interaction between the autokinase EpsE and EpsL in the cytoplasmic membrane is required for extracellular secretion in *Vibrio cholerae*.** *EMBO J* 1995, **14**:1664-1673.

49. Py B, Loiseau L, Barras F: **Assembly of the type II secretion machineryof *Erwinia chrysanthemi*: direct interaction and associated conformational change between OutE, the putative ATP-binding component and the membrane protein OutL.** *J Mol Biol* 1999, **289**:659-670.

50. Possot OM, Pugsely AP: **The conserved tetracysteine motif in the general secretory pathway component PulE is required for efficient pullulanase secretion.** *Gene* 1997, **192**:45-50.

51. Sandkvist M, Hough LP, Bagdasarian MM, Bagdasarian M: **Direct interaction of the EpsL and EpsM proteins of the general secretion apparatus in *Vibrio cholerae*.** *J Bacteriol* 1999, **181**:3129-3135.

52. Bleves S, Gérard-Vincent M, Lazdunski A, Filloux A: **Structure-function analysis of XcpP, a component involved in general secretory pathway-dependent protein secretion in *Pseudomonas aeruginosa*.** *J Bacteriol* 1999, **181**:4012-4019.

53. Possot OM, Gérard-Vincent M, Pugsley AP: **Membrane association and multimerization of secreton component PulC.** *J Bacteriol* 1999, **181**:4004-4011.

54. Galan JE, Bliska JB: **Cross-talk between bacterial pathogens and their host cells.** *Annu Rev Cell Dev Biol* 1996, **12**:221-255.

55. Macnab RM: **Flagella and motility.** In *Escherichia coli and Salmonella: Cellular and Molecular Biology*, 2nd edn. Edited by Neidhardt FC. Washington DC: ASM Press; 1996:123-145.

56. Young GM, Schmiel DH, Miller VL: **A new pathway for the secretion of virulence factors by bacteria: the flagellar export apparatus functions as a protein-secretion system.** *Proc Natl Acad Sci USA* 1999, **96**:6456-6461.

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

58. •• Blocker A, Gounon P, Larquet E, Niebuhr K, Cabiaux V, Parsot C, Sansonetti P: **The tripartite type III secreton of *Shigella flexneri* inserts IpaB and IpaC into host membranes.** *J Cell Biol* 1999, **147**:683-693.
This paper presents evidence that the *Shigella* IpaB and IpaC proteins, homologs of *Yersinia* YopB and YopD, form pores by inserting into the eukaryotic cell plasma membrane. The paper also presents electron micrographs of the *Shigella* type III secretion apparatus, which contains a large bulb region that presumably extends into the bacterial cytoplasm.

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

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

61. • Anderson DM, Schneewind O: ***Yersinia enterocolitica* type III secretion: an mRNA signal that couples translation and secretion of YopQ.** *Mol Microbiol* 1999, **31**:1139-1148.
This paper demonstrates that translation of the YopQ protein is coupled to its secretion by the type III system and presents evidence that an mRNA signal controls YopQ targeting.

62. Cheng LW, Schneewind O: ***Yersinia enterocolitica* type III secretion: on the role of SycE in targeting YopE into HeLa cells.** *J Biol Chem* 1999, **274**:22102-22108.

63. •• Tardy F, Homblé F, Neyt C, Wattiez R, Cornelis GR, Ruysschaert J-M, Cabiaux V: ***Yersinia enterocolitica* type III secretion-translocation system: channel formation by secreted Yops.** *EMBO J* 1999, **18**:6793-6799.
The YopB and YopD proteins of *Yersinia* are shown to be inserted by the type III system into liposomes. Fusion of these liposomes with lipid bilayers results in the formation of channels, dependent on YopB and modified by YopD. Together with [58••], this suggests YopB and YopD and their homologs may form a channel in the target cell membrane for translocation of effector proteins.

64. Neyt C, Cornelis GR: **Insertion of a Yop translocation pore into the macrophage plasma membrane by *Yersinia enterocolitica*: requirement for translocators YopB and YopD, but not LcrG.** *Mol Microbiol* 1999, **33**:971-981.

65. Håkansson S, Schesser K, Persson C, Galyov EE, Rosqvist R, Homble F, Wolf-Watz H: **The YopB protein of *Yersinia pseudotuberculosis* is essential for the translocation of Yop effector proteins across the target plasma membrane and displays a contact-dependent membrane disrupting activity.** *EMBO J* 1996, **15**:5812-5823.

66. Lee VT, Schneewind O: **Type III machines of pathogenic yersiniae secrete virulence factors into the extracellular milieu.** *Mol Microbiol* 1999, **31**:1619-1629.

67. Segal G, Purcell M, Shuman HA: **Host cell killing and bacterial conjugation require overlapping sets of genes within a 22-kb region of the *Legionaella pnumophila* genome.** *Proc Natl Acad Sci USA* 1998, **95**:1669-1674.

68. Weiss AA, Johnson FD, Burns DL: **Molecular characterization of an operon required for pertussis toxin secretion.** *Proc Natl Acad Sci USA* 1993, **90**:2970-2974.

69. Vogel JP, Andrews HL, Wong SK, Isberg RR: **Conjugative transfer by the virulence system of *Legionella pneumophila*.** *Science* 1998, **279**:873-876.

70. Covacci A, Falkow S, Berg DE, Rappuoli R: **Did the inheritance of a pathogenicity island modify the virulence of *Helicobacter pylori*?** *Trends Microbiol* 1997, **5**:205-208.

71. Fernandez D, Dang TAT, Spudich GM, Zhou X-R, Berger BR, Christie PJ: **The *Agrobacterium tumefaciens virB7* gene product, a proposed component of the T-complex transport apparatus, is a membrane-associated lipoprotein exposed at the periplasmic surface.** *J Bacteriol* 1996, **178**:3156-3167.

72. Thorstenson YR, Kuldau GA, Zambryski PC: **Subcellular localization of seven VirB proteins of *Agrobacterium tumefaciens*: implications for the formation of a T-DNA transport structure.** *J Bacteriol* 1993, **176**:5233-5241.

73. Spudich GM, Fernandez D, Zhou X-R, Christie PJ: **Intermolecular disulfide bonds stabilize VirB7 homodimers and VirB7/VirB9 heterodimers during biogenesis of the *Agrobacterium tumefaciens* T-complex transport apparatus.** *Proc Natl Acad Sci USA* 1996, **93**:7512-7517.

74. Christie PJ, Ward JE, Gordon MP, Nester EW: **A gene required for transfer of T-DNA to plants encodes an ATPase with autophosphorylating activity.** *Proc Natl Acad Sci USA* 1989, **86**:9677-9681.

75. Shirasu K, Koukolikova-Nica Z, Hohn B, Kado CI: **An inner-membrane-associated virulence protein essential for T-DNA transfer from *Agrobacterium tumefaciens* to plants exhibits ATPase activity and similarities to conjugative transfer genes.** *Mol Microbiol* 1994, **11**:581-588.

76. Dang TA, Zhou X-R, Graf B, Christie PJ: **Dimerization of the *Agrobacterium tumefaciens* VirB4 ATPase and the effect of ATP-binding cassette mutations on the assembly and function of the T-DNA transporter.** *Mol Microbiol* 1999, **31**:1239-1253.

77. Bohne J, Yim A, Binns AN: **The Ti plasmid increases the efficiency of *Agrobacterium tumefaciens* as a recipient in *virB*-mediated conjugal transfer of an IncQ plasmid.** *Proc Natl Acad Sci USA* 1998, **95**:7057-7062.

78. Lai E-M, Kado CI: **Processed VirB2 is the major subunit of the promiscuous pilus of *Agrobacterium tumefaiens*.** *J Bacteriol* 1998, **180**:2711-2717.

79. Eisenbrandt R, Kalkum M, Lai E-M, Lurz R, Kado CI, Lanka E:
• **Conjugative pili of IncP plasmids, and the Ti plasmid T Pilus are composed of cyclic subunits.** *J Biol Chem* 1999, **274**:22548-22555.
This paper shows that the structural subunits of conjugative and VirB pili are cyclized by a novel intramolecular head-to-tail peptide bond.

80. Sauvonnet N, Vignon G, Pugsley AP, Gounon P: **Pilus formation and protein secretion by the same machinery in *Escherichia coli.*** *EMBO J* 2000, **19**:2221-228.

81. Odenbreit S, Puls J, Sedlmaier B, Gerland E, Fischer W, Haas R: **Translocation of *Heliobacter pylori* CagA into gastric epithelial cells by type IV section.** *Science* 2000, **287**:1497-1500.