Bacterial Pili and Fimbriae

Secondary article

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Bacterial pili and fimbriae are hairlike structures extending from the cell surface that are involved in conjugation, adherence and twitching motility.

Article Contents

Introduction

The terms fimbriae (Latin meaning threads or fibres; singular, fimbria) and pili (Latin meaning hair; singular, pilus) were introduced by Duguid and Brinton, respectively, to describe nonflagellar filamentous appendages of bacteria (Krogfelt, 1991). Presently, both terms are used interchangeably, although many researchers prefer to reserve the term pili for sexual appendages involved in conjugation.

Early studies on bacterial fimbriae and pili were descriptive (see review by Krogfelt, 1991). Researchers began to classify fimbriae based on their differences in morphology, serology or haemagglutination activity. Given the diversity of this group of surface structures, it created a chaotic situation when different classification systems were proposed based on various standards. Brinton, for example, described six types of pili, designated types I–V and F, based on distinct morphology. Meanwhile, Duguid and colleagues proposed a system to type bacterial fimbriae into seven categories, types 1–6 and F, based on different thickness and haemagglutination activity (mannose-resistant or -sensitive). The effort by Ørskov, trying to define the fimbrial adhesins as a fourth group of bacterial surface antigens (F group) and retype fimbriae serologically, failed to establish a generally accepted system. *Escherichia coli* fimbriae K88 and K99 were given K denominations because they were initially wrongly identified as capsular antigens. Because of the confusion created by these different classification systems, newly discovered fimbriae were often simply named after their functions (e.g. CFA for colonization factor antigens), their contribution to certain diseases (e.g. P fimbriae, encoded by *pap* genes, for pyelonephritis-associated pili), their receptors (e.g. S fimbriae bind to sialylgalactoside, and Dr adhesins recognize Dr blood group antigen), their expression patterns (e.g. TCP for toxin-coregulated pili) or their characteristic appearance (e.g. BFP for bundle-forming pili).

Molecular details of bacterial fimbriae biogenesis were elucidated in the 1990s. Genes encoding structural and accessory proteins of various fimbrial systems from diverse species have been isolated and sequenced. With this new information, researchers began to look beyond the morphological diversity and into the common features shared by groups of bacterial fimbriae. Often, a variety of morphologically or serologically different fimbriae were placed in the same group because of their similarities in the assembly machinery or strong homology in the primary amino acid sequences of the structural subunits (Strom and Lory, 1993; Tennent and Mattick, 1994; Hultgren *et al*., 1996). In this section, we classify bacterial pili and fimbriae into three major groups: sex pili, fimbrial adhesins assembled through the chaperone/usher pathway, and type IV fimbriae. Generally, each group is specialized in a distinct function: sex pili in conjugation; fimbrial adhesins in adherence; type IV fimbriae in twitching motility. It should be noted, however, that not all type IV fimbriae are related to twitching motility, and in fact many of them mediate bacterial adherence as well.

Fimbria-like structures, putative adherence factors, were found on surfaces of Gram-positive bacteria, such as *Streptococcus pyogenes* (M protein) and *Actinomyces naeslundii* (type 1 and type 2 fimbriae). However, these fimbria-like structures, unlike Gram-negative bacterial fimbriae, have poorly defined shape. Little is known about how these structures are assembled except that proteins composing them share common features with other surface proteins of Gram-positive bacteria (Dramsi *et al*., 1993). These proteins each has an N-terminal signal peptide, regions of tandem repeats in the middle, and a distinct C-terminal region comprised of a proline/glycine-rich cell wall-spanning domain and a hydrophobic membrane-spanning domain preceded with a hexapeptide LPXTGX and followed by a short, positively charged tail. Gram-positive surface proteins are involved in cell–cell recognition, as is the case for Gram-negative bacteria (Dramsi *et al*., 1993). Given the fundamental differences in cell wall construction between Gram-positive and Gram-negative bacteria, their surface structures are surely assembled through very different mechanisms. Since little is known about the mechanisms by which surface proteins are translocated across the cell wall and assembled into fimbria-like structures in Gram-positive bacteria, it will not be further discussed here.

Sex Pili

Conjugation is a process of DNA transfer from donor to recipient cells that requires close cell–cell contact (see conjugation, below). The presence of an episomal deoxyribonucleic acid (DNA) element, termed a conjugative plasmid, confers on the donor the ability to transfer DNA. DNA transfer in conjugation is a replication process, as a result of which both donor and recipient cells maintain a copy of the transferred DNA. The conjugative plasmid can sometimes integrate into the chromosome through site-specific recombination and facilitate chromosomal DNA transfer, which has become a widely used tool for mapping genetic markers on bacterial chromosomes. In addition, the conjugative plasmid can facilitate transfer of any nonconjugative, mobilizable plasmid coresident in the donor cell. This section is focused on the *E. coli* F pilus and its role in conjugation. (For a broader view of bacterial conjugation see Clewell (1993) and Firth *et al*. (1996).)

Despite the wide application of conjugation as a genetic tool in research since its discovery by Lederberg and Tatum in 1946, little is known about the molecular events that underlie this process, especially at the early stages when physical contacts are established through sex pili produced by the donor cells. The 33.3-kb transfer region (*tra*) that contains genes required for the conjugal transfer of *E. coli* K-12 F (fertility) factor was completely sequenced. Sixteen of the 37 gene products encoded by this region are involved in F pilus biogenesis (Firth *et al*., 1996).

F pili, typically 1–2-μm long filamentous structures extending from the donor cell surface, were distinguished from other fimbriae by adsorption of ribonucleic acid (RNA) phages along them. X-ray diffraction studies revealed each F pilus as a cylindrical filament with an outside diameter of 8 nm and a 2 nm central hydrophilic lumen. Protein subunits (pilins) are arranged in a helical array along the filament axis that contains five subunits per turn, with a rise of 1.28 nm each turn and a repeat distance of 32 nm. In an alternative model, doughnut-like discs each containing five pilins are stacked such that each successive disc is translated 1.28 nm along the filament axis and rotated 28.8°. So far, F pilin has been the only protein subunit detected from the purified F pili, but the existence of possible minor pilins should not be ruled out.

A 121 amino acid precursor of the pilus subunit is encoded by gene *traA*. Processing of the 12.8-kDa prepilin TraA into the 7.2-kDa mature F pilin purified from F pili requires gene products of *traQ* and *traX*. TraQ, a 10.9-kDa cytoplasmic membrane protein, appears to facilitate the rapid insertion of TraA into the cytoplasmic membrane, where its unusually long 51 amino acid signal peptide is cleaved off by signal peptidase I. The expression of *traX* is required for the N^{α}-acetylation of the N-terminal alanine residue of F pilin. Even though N^{α}-acetylation of pilin is found to be common to all the F-like systems characterized so far, it does not seem to be essential for F pilus biogenesis or function. There is evidence suggesting that N-terminal acetylation appears to affect the folding of F pilin, thereby masking certain epitopes: antibodies recognizing unacetylated pili fail to bind wild-type pili; F pili composed of unacetylated pilins form massive aggregates, whereas those composed of acetylated pilins tend to remain isolated.

Even though F pilin is destined to form extracellular appendages, the majority of F pilin was found, surprisingly, associated with the cytoplasmic membrane. F pilin contains four structural domains: a hydrophilic N-terminal domain I, followed by two hydrophobic domains II and IV that are separated by a basic KNVK tetrapeptide as domain III. The topology of F pilin in the cytoplasmic membrane has been revealed by *traA'-'phoA* gene fusions (Paiva *et al*., 1992). The two hydrophobic transmembrane domains II and IV are oriented such that domain III remains in the cytoplasm and domain I extends into the periplasm. There appears to be a pool of F pilin in the outer membrane as well. Immunogold labelling using an F pilin monoclonal antibody (Paiva *et al*., 1992) has shown that, while most cytoplasmic membrane F pilins remain separate, some outer membrane F pilins exist in aggregates as possible assembly or disassembly intermediates. It is not yet clear how the cytoplasmic or outer membrane F pilin pool is related to the F pilus in terms of dynamics. Mutations in *traL*, *traE*, *traK*, *traB*, *traV*, *traC*, *traW*, *traU*, *traF*, *traH*, *traG*, *trbC*, or *traI* appear to affect F pilus biogenesis (as summarized by Firth *et al*., 1996). Little is known about the functions of these gene products other than that all of them are, directly or indirectly, associated with the cell envelope (cytoplasmic membrane, periplasm, or outer membrane). Further studies are required to determine how F pilus is assembled through this multi-component machinery.

For a long time, the role of F pilus in conjugation was believed to be in providing a channel for the passage of DNA from donor to recipient. However, the observation of electron-dense conjugation junctions formed between conjugating bacteria suggests that a more intimate and stabilized cell–cell contact occurs during the process. F pilus appears to dock donor and recipient cells and retract to bring them into close physical contact to form conjugation junctions. Given the unknown identity of these conjugation junctions, it is possible that the disassembled F pilus or a part of its assembly machinery may play structural roles in the establishment of conjugation junctions.

Fimbrial Adhesins and the Chaperone/Usher Pathway

The first step in colonization of a host by a microbe often requires specific adherence to host receptors. Pathogenic

bacteria produce adhesive proteins (adhesins) that help themselves attach to the host surface to overcome cleansing mechanisms such as sneezing, coughing and constant fluid flow in gastric or urinary tracts (Beachey, 1981). Many adhesins are presented at tips of fimbrial structures that are composed of major and minor pilins. There are adhesins that are not associated with any fimbrial structures as well, known as afimbrial adhesins or nonpilus-associated adhesins, such as the afimbrial adhesins (AFAI and AFAIII) of uropathogenic and diarrhoea-associated *E. coli*. Some adhesins are referred to as haemagglutinins because of their ability to agglutinate erythrocytes, owing to the presence of their receptors on surfaces of red blood cells. Based on whether or not their haemagglutination activities can be inhibited by mannose, haemagglutinins are divided into two categories: the mannose-sensitive and the mannose-resistant. Most of the receptors identified thus far are carbohydrate moieties of glycolipids or glycoproteins present on the host cell membrane, or components of extracellular matrix such as fibronectin, type IV collagen, laminin and plasminogen (Hultgren *et al*., 1996). Bacterial adhesins and their host receptors are crucial factors in determining the host specificity and tissue tropism of pathogens. The critical contribution of bacterial adhesins to pathogenesis, especially at the early stages of disease, has been demonstrated in a variety of pathogens, including uropathogenic, enteropathogenic and enterotoxigenic *E. coli*, *Proteus mirabilis*, *Salmonella typhimurium*, *Bordetella pertussis*, *Haemophilus influenzae*, *Klebsiella pneumoniae*, *Neisseria gonorrhoeae*, *Pseudomonas aeruginosa* and others (Strom and Lory, 1993; Thanassi *et al*., 1998). Due to changes in environment during the course of infection, some pathogenic strains are adapted to express a variety of adhesins specialized in binding to different host cells. For example, uropathogenic *E. coli* express type 1 fimbriae and P fimbriae that bind to bladder and kidney epithelial surfaces, respectively.

Fimbrial adhesins can be divided into two groups according to their differences in the mechanisms of biogenesis (Hultgren *et al*., 1996; Thanassi *et al*., 1998). The majority of adhesins, whether fimbrial or afimbrial, adopt the chaperone/usher pathway to transport pilus subunits from the cytoplasmic membrane to the outer membrane. The other group of adhesins, known as type IV (or type 4) fimbriae, are assembled through a multicomponent machinery that shares strong homology to the type II secretion system but, as yet, is not fully characterized (Thanassi *et al*., 1998). Type IV fimbriae will be discussed in the next section.

The chaperone/usher system has been characterized by Hultgren's group through extensive studies on *E. coli* P fimbriae. P fimbria, encoded by pap genes, is an adhesive organelle produced by uropathogenic *E. coli* that binds to the Galα(1–4)Gal moieties of the globoseries of glycolipids present on surfaces of kidney cells and erythrocytes. The tip adhesin, PapG, is joined by an adaptor, PapF, to a 2-nm diameter fibrillum comprised of PapE, which is then joined by another adaptor, PapK, to the distal end of a 6.8-nm diameter helical rod that is comprised of PapA and anchored to the outer membrane by PapH (**Figure 1**; Thanassi *et al*., 1998). Newly synthesized pilin subunits, including PapG, PapF, PapE, PapK, PapA and PapH, are translocated across the plasma membrane through the traditional *sec*-dependent pathway involved in the type II secretion system. Efficient release of pilin subunits from the plasma membrane requires periplasmic chaperone protein PapD, which then escorts them to the assembly site in the outer membrane usher PapC (Hultgren *et al*., 1996). In addition to PapD, the periplasmic disulfide isomerase DsbA is also required for correct folding of pilin subunits.

The crystal structure of the chaperone PapD and a 19 amino acid C-terminal peptide of PapG revealed that the peptide lies in the cleft between the two immunoglobulin domains of PapD and interacts specifically with the G1 β strand to form a β-sheet structure, termed a β zipper. Therefore, the C-terminal chaperone-binding domain of pilin subunits is also referred to as a β-zipper motif. The periplasmic chaperone family is divided into two subfamilies based on their structural differences, especially the difference in the length of the loop that connects the F1 and G1 β strands (Thanassi *et al*., 1998). The significance of these differences in fimbrial assembly is clearly indicated by the fact that chaperones from the two subfamilies mediate the assembly of two distinct fimbrial structures. Members of the FGL chaperone subfamily have a long F1 to G1 loop and mediate the assembly of rod-like fimbriae, whereas members of the FGS subfamily have a short F1 to G1 loop and mediate the assembly of either afimbrial adhesins or adhesins with very fine threads or amorphorous structures.

Periplasmic chaperone-pilin subunit complexes are then targeted to the outer membrane usher protein, where the pilin subunits are dissociated from chaperones, translocated across the outer membrane and assembled into fibre structures. Studies by Thanassi *et al*. showed that purified PapC forms pores when reconstituted into liposomes. High-resolution electron micrographs indicated that PapC assembles into 15-nm diameter ring-shaped complexes with 2–3-nm diameter pores in the centre (Thanassi *et al*., 1998). Since the 2–3-nm diameter channel in the multimeric PapC complex is only wide enough for the translocation of the 2-nm diameter tip fibrillum, it is hypothesized that the 6.8-nm diameter PapA rod is also translocated across the outer membrane as an extented 2-nm diameter fibre, which is then wound into a helical rod after passage through the channel. The hypothesis is supported by experimental observations that, under certain conditions, the pilus shaft can be unravelled into 2-nm diameter linear fibres (**Figure 1**; Thanassi *et al*., 1998). In addition to its structural role of forming a channel in the outer membrane, PapC is believed to play a more active role in fimbrial biogenesis. The differential affinities between PapC and various PapD–pilin subunit complexes

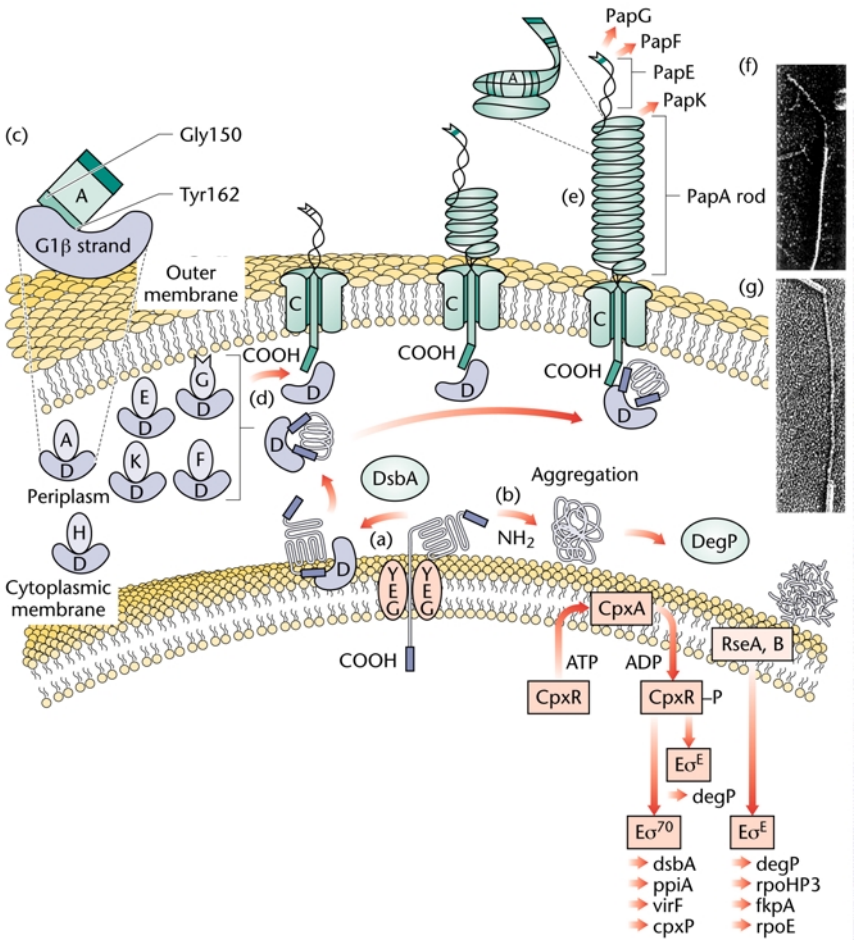

Figure 1 A model for the biogenesis of P pili. (a) Subunits (PapA, E, F, G, H, K) are translocated across the cytoplasmic membrane by the Sec machinery and they interact sequentially with the periplasmic disulfide isomerase DsbA and the chaperone PapD. DsbA mediates disulfide bond formation in the subunits and PapD, and it is required for the correct folding of PapD. PapD is needed for the release of subunits from the cytoplasmic membrane and for the proper folding of the subunits. (b) In the absence of PapD, subunits enter into nonproductive aggregations that are sensed by the Cpx and σE signal transduction pathways. These pathways activate a number of genes including the gene that encodes the DegP protease which degrades the subunit aggregates. (c) The chaperone binds, in part, to a conserved C-terminal motif of the subunits which consists of a conserved glycine (Gly150) followed by a pattern of alternating hydrophobic residues, including a conserved penultimate tyrosine (Tyr162). This motif makes a β-zipper interaction with the G1 β strand of PapD. (d) Chaperone–subunit complexes are targeted to PapC in the outer membrane, where subunit–subunit interactions lead to the formation and translocation of a linear pilus fibre across the outer membrane through the usher channel. (e) Once on the cell surface, the pilus rod can twist into its final helical conformation, which may facilitate secretion of the pilus. On the top right (f) is a high-resolution electron micrograph showing the two subassemblies of the P pilus: the tip fibrillum and pilus rod. Below (g) is a high-resolution electron micrograph showing the unravelling of a portion of a P pilus rod into a linear fibre. (Reprinted with permission from Thanassi *et al.*, 1998.)

may be crucial factors in determining their final position in the pilus (Thanassi *et al.*, 1998).

Unlike the assembly machinery for sex pili or type IV fimbriae, the chaperone/usher system has no nucleotide-binding components. The fimbriae assembly across the outer-membrane usher is adenosine triphosphate (ATP)-independent. It is the winding of the PapA fibre into a helix on the surface of bacteria, combined with the affinity of chaperone–pilin complexes for the usher and the polymerization of pilins, that provides the driving force for the translocation of fimbriae across the outer membrane (Thanassi *et al.*, 1998).

Type IV Fimbriae and Twitching Motility

Unlike fimbriae assembled through the chaperone/usher pathway, which are usually arranged peritrichously around the bacterial cell, type IV fimbriae are commonly

located at the poles of bacterial cells. Type IV fimbriae have been identified as key host colonization factors in a wide range of pathogenic bacteria, including *Pseudomonas aeruginosa*, *Moraxella bovis*, *Dichelobacter nodosus*, *Neisseria meningitidis*, *Neisseria gonorrhoeae*, *Vibrio cholerae* and enteropathogenic *E. coli* (Strom and Lory, 1993; Tennent and Mattick, 1994). In addition to the adhesive properties common to most fimbrial structures, in some species type IV fimbriae are associated with a form of movement on solid surfaces, termed twitching motility (Henrichsen, 1983). The physical basis of twitching motility has been proposed to rely on the ability of type IV pili to undergo reversible extension and retraction. Even though this is currently a widely accepted model, there is as yet no direct evidence supporting it.

Type IV fimbriae are related through their similarities in many respects, including the primary amino acid sequence of the structural subunits, the proteolytic processing and *N*-methylation of the prepilins, the conserved assembly machinery, and, to a lesser extent, the mechanisms of transcriptional regulation (Strom and Lory, 1993; Tennent and Mattick, 1994). As a common feature for surface structures of pathogenic bacteria, some type IV fimbriae undergo phase variation that may allow for the successful escape from the host immune system. The mechanisms of transcriptional regulation of type IV fimbriae will be discussed in the next section, along with the other mechanisms adopted by different fimbrial systems to undergo phase variation.

Type IV fimbriae are mostly composed of a single structural subunit (pilin), although tip adhesins were identified in *N. gonorrhoeae* type IV fimbria. Type IV pilins of different species share several distinctive features, including a short, positively charged leader peptide ending with a glycine residue in the precursor (prepilin), *N*-methylation of the first amino acid residue in the mature protein (pilin), and a highly conserved hydrophobic N-terminal region referred to as the constant domain (Strom and Lory, 1993; Tennent and Mattick, 1994). The fimbrial subunits of the bundle-forming pilus of enteropathogenic *E. coli* and the toxin-coregulated pilus of *V. cholerae*, BfpA and TcpA, respectively, are placed in a subgroup of type IV pilins (group B) due to minor differences in those features. The C-terminal two-thirds of the pilin, referred to as the variable domain, provides the basis for antigenic variation of type IV fimbriae from different species, and in the case of *N. gonorrhoeae*, it is also the source of antigenic variation occurring within the same strain by gene conversion.

Given the similarities in the structural subunits, type IV fimbriae are speculated to be assembled through a common machinery. Indeed, this is supported by the fact that *P. aeruginosa* is capable of producing type IV fimbriae of *D. nodosus*, *M. bovis* and *N. gonorrhoeae* from cloned fimbrial subunits. Unlike fimbriae assembled through the chaperone/usher pathway, where genes responsible for fimbrial biogenesis are clustered near the structural genes, genes required for type IV fimbrial biogenesis are scattered all over the chromosome, with the exception of Tcp and Bfp, the two members of group B type IV fimbriae family (Strom and Lory, 1993; Tennent and Mattick, 1994). Genes involved in production of Tcp fimbriae are mostly organized as a gene cluster on the chromosome, whereas the cluster of 14 genes that are sufficient for the biogenesis of Bfp fimbriae is located on a plasmid.

Transposon mutagenesis studies have identified a large number of genes involved in type IV fimbrial biogenesis and twitching motility of *P. aeruginosa* (summarized in Alm and Mattick, 1997). This encompasses genes required for the processing of prepilins, genes involved in the transcriptional regulation, and for the most part, genes with unknown functions that are clearly required for fimbriae assembly.

The leader peptidase and *N*-methyl-transferase activities for processing of PilA, the prepilin of *P. aeruginosa* type IV fimbria, are both properties of an inner membrane protein, PilD. Mutations in *pilD* lead to complete loss of surface fimbriae and accumulation of unprocessed prepilins inside bacterial cells. The proteolytic cleavage and *N*-methylation of prepilin has been reconstituted *in vitro* using purified PilD, purified prepilin and *S*-adenosyl-L-methionine as the methyl donor (Strom and Lory, 1993). Additionally, purified PilD from *P. aeruginosa* could cleave the leader peptide off the prepilin of *N. gonorrhoeae*. The *N*-methyltransferase activity of PilD is likely to be conserved in all type IV prepilin peptidases, given the same modification that all their mature pilins have. The periplasmic disulfide isomerase DsbA is required for proper folding of BfpA, as is the case for PapA (Thanassi *et al*., 1998).

Among the gene products that are required for the twitching motility of *P. aeruginosa*, there is a set of proteins that are homologous to *E. coli* chemotaxis proteins, including PilG (CheY), PilH (CheY), PilI (CheW), PilK (CheR), PilL (CheA), PilJ (MCP, methyl-accepting chemotaxis proteins). It is proposed that these proteins transmit environmental signals to PilB and PilT/PilU, putative nucleotide-binding proteins, which in turn may provide energy to a yet to be identified motor system to extend or retract type IV fimbriae, and thereby undergo twitching movement.

In addition to PilD, there are other gene products involved in the fimbriae assembly process in the cell envelope. These gene products include an outer membrane protein PilQ, a cytoplasmic membrane protein PilC, proteins containing type IV leader sequences (PilE and PilV), and others. DNA sequencing of regions between the loci identified through transposon mutagenesis studies has identified more genes involved in type IV fimbrial biogenesis (Alm and Mattick, 1997). Even though little is known about the specific function of each individual, the extensive amino acid sequence analysis of these gene products has revealed strong homology to components involved in protein secretion, DNA uptake, and filamen-

tous phage assembly in a variety of microorganisms, suggesting deep functional and evolutionary links between these systems (Hobbs and Mattick, 1993). The connection between type IV fimbrial assembly and DNA uptake is even closer in *N. gonorrhoeae* where production of type IV fimbriae is a prerequisite for natural competence for DNA uptake and transformation, and, on the other hand, DNA transformation-mediated horizontal gene exchange is crucial for the antigenic variation of the major pilin PilE.

Phase Variation

Clearly, fimbrial adhesins are important virulence factors for pathogenic bacteria. However, because of their surface localization, proper temporal and spatial expression of fimbrial structures is not only crucial for their function as adhesins but also very important for evading the host immune system. Bacterial two-component sensor-regulator systems play an essential role in gene regulation in response to environmental cues. One such system in regulating fimbrial gene expression is the *P. aeruginosa* PilS-PilR system (Strom and Lory, 1993; Tennent and Mattick, 1994). PilS, a cytoplasmic membrane protein, senses and transmits environmental signals to a cytoplasmic DNA-binding protein, PilR, through a series of phosphorylation reactions. Mutations in *pilS* or *pilR* result in nonpiliated mutants. The presence of a RpoN-interaction domain in PilR suggests that PilR may affect the RpoN(σ^{54})-dependent transcription of the pilin gene, *pilA*. The environmental signal recognized by PilS and the immediate target of PilR are still unknown. The RpoN(σ^{54})-dependent expression is conserved in all members of the group A type IV pilin family. Expression of Tcp fimbriae is regulated by the ToxR/ToxS/ToxT system, which also controls the transcription of cholera toxin (Strom and Lory, 1993).

A variety of molecular events are involved in the transcriptional regulation of fimbrial gene clusters. Site-specific recombination mediated by FimB and FimE is reponsible for the inversion of a DNA fragment that contains the promoter for transcription of type I fimbrial gene cluster (*fim*) of *E. coli* (Klemm, 1986). A similar mechanism is involved in the regulation of MR/P fimbriae production in *P. mirabilis* (Zhao *et al*., 1997). DNA methylation plays a critical role in the transcriptional regulation of the P fimbrial gene cluster (*pap*) of *E. coli*, which involves the leucine response protein (LRP), catabolite activator protein (CAP), histone-like protein (H-NS), and *pap*-encoded PapB and PapI (van der Woude *et al*., 1996).

However, not all phase variation occurs at the transcriptional level. The phase-variable expression of *N. gonorrhoeae* type IV fimbrial adhesin, PilC, relies on slipped-strand mispairing, a process that occurs during DNA replication of regions containing highly repetitive DNA sequences (Seifert, 1996). The *pilC* gene contains a run of Gs near the beginning of the coding region. Frameshifts resulting from insertion or deletion of G residues by slipped-strand mispairing determine whether or not PilC will be expressed as an intact protein. The tRNA for a rare leucine codon (UUG), encoded by *leuX*, appears to stimulate type 1 fimbrial expression, presumably by increasing the expression of FimB, which contains six leucine residues encoded by UUG (Newman *et al*., 1994).

In addition to phase variation, antigenic variation of fimbrial structural subunits is another common mechanism adopted by pathogenic bacteria to avoid attack by host immune system. One such example is the antigenic variation of the type IV pilin of *N. gonorrhoeae*, PilE, which results from the gene conversion between one of several silent pilin genes (*pilS*) and the expressed gene (*pilE*) (Seifert, 1996).

Concluding Remarks

Despite the tremendous diversity of bacterial fimbriae with respect to morphology, assembly, genetics and regulation, certain features unite this group of structures. Extending from the surface of cells, fimbriae are able to overcome the forces of cell-to-cell repulsion caused by the negative charges on their respective surfaces. Fimbriae allow cell-to-cell contact, whether it be interaction of a bacterium with another bacterium or a bacterium with its eukaryotic target cell. Specific adhesin–receptor interactions or nonspecific hydrophobic interactions can mediate these attachments. Bacteria have devised extraordinary strategies to assemble these complex structures outside the cell, using mechanisms that are independent of the traditional cytoplasmic energy sources. While some systems are well understood, it is clear that much work remains to elucidate mechanisms of assembly and function of many fimbrial systems.

References

Alm RA and Mattick JS (1997) Genes involved in the biogenesis and function of type-4 fimbriae in *Pseudomonas aeruginosa*. *Gene* **192**: 89–98.

Beachey EH (1981) Bacterial adherence: adhesin–receptor interactions mediating the attachment of bacteria to mucosal surfaces. *Journal of Infectious Diseases* **143**: 325–344.

Clewell DB (ed.) (1993) *Bacterial Conjugation*. New York: Plenum.

Dramsi S, Dehoux P and Cossart P (1993) Common features of Gram-positive bacterial proteins involved in cell recognition. *Molecular Microbiology* **9**: 1119–1122.

Firth N, Ippen-Ihler K and Skurray R (1996) Structure and function of the F factor and mechanism of conjugation. In: Neidhardt FC (ed.) Escherichia coli *and* Salmonella*: Cellular and Molecular Biology*. Washington, DC: ASM Press.

Henrichsen J (1983) Twitching motility. *Annual Review of Microbiology* **37**: 81–93.

Hobbs M and Mattick JS (1993) 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. *Molecular Microbiology* **10**: 233–243.

Hultgren SJ, Jones CH and Normark S (1996) Bacterial adhesins and their assembly. In: Neidhardt FC (ed.) Escherichia coli *and* Salmonella*: Cellular and Molecular Biology*. Washington, DC: ASM Press.

Klemm P (1986) Two regulatory *fim* genes, *fimB* and *fimE*, control the phase variation of type 1 fimbriae in *Escherichia coli*. *EMBO Journal* **5**: 1389–1393.

Krogfelt KA (1991) Bacterial adhesion: genetics, biogenesis, and role in pathogenesis of fimbrial adhesins of *Esherichia coli*. *Reviews of Infectious Diseases* **13**: 721–735.

Newman JV, Burghoff RL, Pallesen L *et al.* (1994) Stimulation of *Escherichia coli* F-18Col- type-1 fimbriae synthesis by *leuX*. *FEMS Microbiology Letters* **122**: 281–287.

Paiva W, Grossman T and Silverman P (1992) Characterization of F-pilin as an inner membrane component of *Escherichia coli* K12. *Journal of Biological Chemistry* **267**: 26191–26197.

Seifert HS (1996) Questions about gonococcal pilus phase- and antigenic variation. *Molecular Microbiology* **21**: 433–440.

Strom MS and Lory S (1993) Structure-function and biogenesis of the type IV pili. *Annual Review of Microbiology* **47**: 565–596.

Tennent JM and Mattick JS (1994) Type-4 fimbriae. In: Klemm P (ed.) *Fimbriae: Aspects of Adhesion, Genetics, Biogenesis and Vaccines*. Boca Raton, FL: CRC Press.

Thanassi DG, Saulino ET and Hultgren SJ (1998) The chaperon/usher pathway: a major terminal branch of the general secretory pathway. *Current Opinion in Microbiology* **1**: 223–231.

van der Woude M, Braaten B and Low D (1996) Epigenetic phase variation of the *pap* operon in *Escherichia coli*. *Trends in Microbiology* **4**: 5–9.

Zhao H, Li X, Johnson DE, Blomfield I and Mobley HL (1997) *In vivo* phase variation of MR/P fimbrial gene expression in *Proteus mirabilis* infecting the urinary tract. *Molecular Microbiology* **23**: 1009–1019.

Further Reading

Clewell DB (ed.) (1993) *Bacterial Conjugation*. New York: Plenum.

Hultgren SJ, Jones CH and Normark S (1996) Bacterial adhesins and their assembly. In: Neidhardt FC (ed.) Escherichia coli *and* Salmonella*: Cellular and Molecular Biology*. Washington, DC: ASM Press.

Klemm P (ed.) (1994) *Fimbriae: Aspects of Adhesion, Genetics, Biogenesis and Vaccines*. Boca Raton, FL: CRC Press.

Strom MS and Lory S (1993) Structure-function and biogenesis of the type IV pili. *Annual Review of Microbiology* **47**: 565–596.

Thanassi DG, Saulino ET and Hultgren SJ (1998) The chaperon/usher pathway: a major terminal branch of the general secretory pathway. *Current Opinion in Microbiology* **1**: 223–231.