

## FimC is a periplasmic PapD-like chaperone that directs assembly of type 1 pili in bacteria

C. HAL JONES\*, JEROME S. PINKNER\*, ANDY V. NICHOLS\*, LYNN N. SLONIM\*, SOMAN N. ABRAHAM\*†, AND SCOTT J. HULTGREN\*‡

\*Department of Molecular Microbiology, Box 8230, Washington University School of Medicine, St. Louis, MO 63110; and †Department of Pathology and Medicine, Jewish Hospital of St. Louis, St. Louis, MO 63110

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**ABSTRACT** Biogenesis of the type 1 pilus fiber in *Escherichia coli* requires the product of the *fimC* locus. We have demonstrated that FimC is a member of the periplasmic chaperone family. The deduced primary sequence of FimC shows a high degree of homology to PapD and fits well with the derived consensus sequence for periplasmic chaperones, predicting that it has an immunoglobulin-like topology. The chaperone activity of FimC was demonstrated by purifying a complex that FimC forms with the FimH adhesin. A *fimC1* null allele could be complemented by the prototype member of the chaperone superfamily, PapD, resulting in the production of adhesive type 1 pili. The general mechanism of action of members of the chaperone superfamily was demonstrated by showing that the ability of PapD to assemble both P and type 1 pili was dependent on an invariant arginine residue (Arg-8), which forms part of a conserved subunit binding site in the cleft of PapD. We suggest that the conserved cleft is a subunit binding feature of all members of this protein family. These studies point out the general strategies used by Gram-negative bacteria to assemble adhesins into pilus fibers, allowing them to promote attachment to eukaryotic receptors.

Periplasmic chaperones are part of a general secretory pathway required for the assembly of several well-characterized extracellular proteinaceous fibers, referred to as fimbriae or pili, in *Escherichia coli*, *Salmonella typhimurium*, *Salmonella enteritidis*, *Haemophilus influenzae*, *Bordetella pertussis*, *Yersinia pestis*, and *Klebsiella pneumoniae* (1–4). The best characterized and prototype member of the periplasmic chaperone superfamily is PapD from the P pilus system (1–9). Periplasmic chaperones stabilize pilus subunits in the periplasm through the formation of distinct periplasmic complexes (6, 8). The ability of chaperones to bind to and cap interactive surfaces on the pilus subunits prevents aggregation of the subunits and allows correct folding and assembly (6, 8).

The crystal structure of PapD has been solved to 2.0 Å resolution and revealed that PapD is composed of two domains each having an overall topology identical to an immunoglobulin fold (ref. 7; D. Ogg, personal communication). The two domains are connected by a hinge region and oriented such that a cleft is created between the two domains (7). Recent work by Slonim *et al.* (9) suggests that invariant surface-exposed residues that protrude into the cleft make up the subunit binding pocket of PapD. In contrast to cytoplasmic chaperones such as GroEL, DnaK, DnaJ, and SecB, which maintain their targets in highly unfolded conformations (10), PapD maintains target proteins in native-like conformations (8). In addition, periplasmic chaperones have an effector function, specifically targeting the subunits to outer membrane assembly sites for their incorporation into pili (11).

Type 1 pili are produced by nearly all Enterobacteriaceae (12). The major component of type 1 pili is repeating FimA subunits arranged in a right-handed helix to form a 7-nm-wide fiber with an axial hole (12). FimF, FimG, and FimH are three minor proteins associated with type 1 pili (13–15). FimH is the mannose-binding adhesin that promotes the interaction of type 1 pilated bacteria with mannose-containing glycoproteins on eukaryotic cell surfaces (14, 16). Type 1 pilus biogenesis requires two genes encoding nonstructural components of the pilus, which were originally identified as *pilB* and *pilC* (17). P pili, on the other hand, are produced specifically by pyelonephritic *E. coli* and mediate binding to Gal( $\alpha$ 1-4)Gal-containing receptors on epithelial cell surfaces (1, 2). PapA is the major component of the P pilus rod while PapE, PapF, PapG, and PapK make up architecturally distinct fibers called tip fibrillae that are joined end-to-end to the pilus rods (18, 19). PapG is the Gal( $\alpha$ 1-4)Gal binding adhesin, and presentation of this moiety in the fibrillum probably promotes efficient interaction with host receptors (1, 2). Assembly of the composite P pilus requires an immunoglobulin-like periplasmic chaperone and an outer-membrane usher, the products of the *papD* and *papC* genes, respectively (1, 2, 5, 11). As reported here, the FimC chaperone was found to have a similar structure, function, and mechanism of action as PapD. We suggest that all PapD-like chaperones utilize their immunoglobulin-like domains in a recognition paradigm to bind to pilus subunit proteins.

### MATERIALS AND METHODS

**Bacterial Strains.** ORN103 (17) was used as a host in all complementation studies. HB101 (20) was used as a background strain for FimC purification. Induction of the *Pap* operon was as described (9). Induction of the type 1 operon was by three 48-hr static passages in Luria broth. Isopropyl  $\beta$ -D-thiogalactoside (IPTG) was used at 5  $\mu$ M in liquid and 10  $\mu$ M in solid media.

**Hemagglutination Assay.** Assays were performed following published protocols (9, 21). Soluble D-mannose (Sigma) at 0.1–0.5% was used to demonstrate mannose-sensitive hemagglutination (MSHA) while soluble Gal( $\alpha$ 1-4)Gal at 0.1 mM was used to demonstrate specific hemagglutination by P pili.

**Genetic Constructs.** pPAP5 (5), pPAP37 (5), pPAP43 (22), pLS101 (9), pRT4 (23), and pJP1 (11) have been described. pJP3 is a pUC18 construct containing the complete type 1 operon cloned as a *Sal* I fragment from pSH2 (19). pJP5 contains an *Xho* I linker in the unique *Eco*RI site in pJP3, which defines the *fimC1* mutation. pJP4 is an *Eco*RI/*Hind*III subclone from pSJH9 (21) into the *tac* promoter plasmid pMMB91 (11). Sequencing of the *fimC* open reading frame<sup>§</sup>

Abbreviations: IPTG, isopropyl  $\beta$ -D-thiogalactoside; MSHA, mannose-sensitive hemagglutination.

<sup>†</sup>To whom reprint requests should be addressed.

<sup>§</sup>The *fimC* sequence reported in this paper has been deposited in the GenBank data base (accession no. L14598).

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with Sequenase (United States Biochemical) followed manufacturer's specifications.

**Purification of FimC and Type 1 Pili.** FimC was purified from the periplasm of HB101 containing pJP4. Bacteria were grown in a 5-liter fermentor (New Brunswick Scientific, Edison, NJ) to an  $A_{600} = 1.0$  at which time IPTG was added to a final concentration of 1 mM. Induction was allowed to proceed for 1 hr, and then periplasm was prepared as described (9). Further purification of FimC by FPLC and HPLC was by a published protocol (5). Purified FimC protein was sent to Cocalico Biologicals (Reamstown, PA) for antibody production in rabbits. Electrophoresis of protein preparations was according to manufacturer's specifications (Bio-Rad). Western blot analyses followed published procedures (9). Purification and quantification of pili from equivalent gram quantities of cultures were as described (9).

**Isolation of a FimC-FimH Complex.** After induction with IPTG for 1 hr, periplasm from ORN103/pJP4 (*fimC*)/pRT4 (*fimH*) was prepared as described (9), dialyzed against phosphate-buffered saline and concentrated on a Centrprep column (Amicon) to 1.5 ml. Nine hundred microliters of the concentrated periplasm was mixed with 100  $\mu$ l of washed mannose-Sepharose beads, rocked overnight, and then washed extensively with phosphate-buffered saline to remove unbound material. The FimC-FimH complex was eluted with 200  $\mu$ l of 10% D-mannose or 10% methyl  $\alpha$ -D-mannopyranoside (Sigma).

## RESULTS

**FimC Is an Immunoglobulin-Like Pilus Chaperone.** Orn-dorff and Falkow (17) demonstrated that the product of the *fimC* gene is required for the assembly of type 1 pili by showing that a mutation in the *fimC* locus resulted in a nonpiliated phenotype (17). In addition, Hultgren *et al.* (21) found that the product of the *fimC* locus was required for the assembly of the mannose-binding adhesin on the surface of bacteria in the absence of the pilus rod. We have cloned (Table 1) and determined the complete nucleotide sequence (data not shown) of both strands of the *fimC* locus from the *E. coli* strain 149 that is a voided urinary isolate from a woman with cystitis (21, 24). The structural relationship between FimC and the superfamily of periplasmic chaperones was investigated by comparison of the predicted amino acid sequence of FimC and the chaperone consensus sequence (Fig. 1) (3, 4). This alignment showed that FimC was 32% identical and 50% homologous, considering conservative

substitutions, to the prototype member of the immunoglobulin-like pilus chaperone family, PapD (Figs. 1 and 2). FimC contains all of the conserved residues that make up the hydrophobic core of pilus chaperones (Fig. 1); these residues participate in maintaining the overall structure of the two domains of the chaperone protein (3). FimC was also shown to possess the invariant internal salt bridge that has been proposed to be important in orienting the two domains of the chaperone with respect to one another (3). FimC also contains two invariant cleft residues (Arg-8 and Lys-112) that were shown by Slonim *et al.* (9) and M. Kuehn, D. Ogg, J. Kilberg, L.N.S., T. Bergfors, K. Flemmer, and S.J.H. (unpublished results) to make up a critical part of the subunit binding site of PapD (Figs. 1 and 2).

The biochemical properties of FimC were also found to be characteristic of periplasmic pilus chaperones. Induction of *fimC* expression, cloned downstream of the *tac* promoter on pJP4 (Table 1), with IPTG resulted in the presence of a 26-kDa protein in the periplasmic space (Fig. 3A, lanes 1 and 2). When periplasmic extracts containing the induced 26-kDa protein were passed over a Mono S FPLC column, the FimC protein was eluted (95% homogeneity) in 0.15 M KCl (Fig. 3A, lane 3). FimC was purified to homogeneity by chromatography on a hydrophobic interaction column, CAA-HIC (Beckman), with conditions similar to that used to purify PapD for crystallography (data not shown). The amino-terminal sequence of the mature form of FimC was obtained, verifying that the induced 26-kDa band is the product of the FimC locus. FimC is a highly basic protein having an isoelectric point of 9.4 (data not shown). All of these properties are characteristic of periplasmic pilus chaperones (3, 8, 9).

**Isolation of a FimC-FimH Complex.** The chaperone activity of FimC was shown by demonstrating that FimC binds FimH, the type 1 adhesin, to form a periplasmic preassembly complex. Since FimH directs binding to receptors that contain mannose derivatives (14, 16, 23), mannose-Sepharose chromatography was used to purify a FimC-FimH complex

Table 1. Bacterial strains and plasmids used

Strain or plasmid	Genotype/remarks	Reference
<b>Strain</b>		
ORN103	<i>thr-1 leu-6 thi-1</i> $\Delta$ ( <i>argF-lac</i> ) <i>U169</i> <i>xyl-7 ara-13 mtl-2 gal-6 rpsL</i> <i>tonA2 minA minB recA13</i> $\Delta$ <i>fim(EACDFGH)</i>	19
HB101	F <sup>-</sup> <i>hsdS20</i> ( $r^{-}_b, m^{+}_k$ ) <i>recA13 ara-14</i> <i>proA2 lacY1 galk2 rpsL20</i> (Sm <sup>r</sup> ) <i>xyl-5 mtl-1 supE44</i>	22
<b>Plasmid</b>		
pPAP5	<i>Pap</i> operon, wild type	5
pPAP37	<i>papD1</i> , <i>Xho</i> I linker insertion	5
pLS101	<i>papD</i> , IPTG inducible	9
pJP1	<i>papD</i> , <i>papG</i> , IPTG inducible	12
pPAP43	<i>P<sub>pap</sub></i> , <i>papIBA</i>	24
pJP3	Type 1 operon, wild type	This study
pJP5	<i>fimC1</i> , <i>Xho</i> I linker insertion	This study
pJP4	<i>fimC</i> , IPTG inducible	This study
pRT4	<i>fimH</i> , IPTG inducible	25

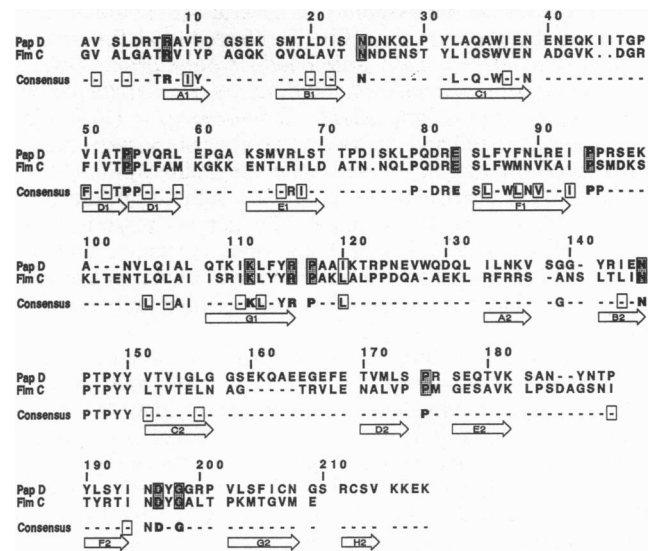


FIG. 1. The primary sequence of PapD and the inferred primary sequence of FimC are shown along with the chaperone consensus sequence, which was derived from 13 members of the family (3, 4). Open boxes in the consensus sequence indicate positions of conserved hydrophobic character. Boldfaced letters in the consensus sequence and stippled boxes in the PapD and FimC sequences indicate invariant residues while residues that are conserved in 8 out of 13 sequences are indicated in the consensus in normal type. Open arrows below the consensus sequence indicate the  $\beta$  strands identified on the crystal structure of PapD (7). This alignment is based on previously published data (4).

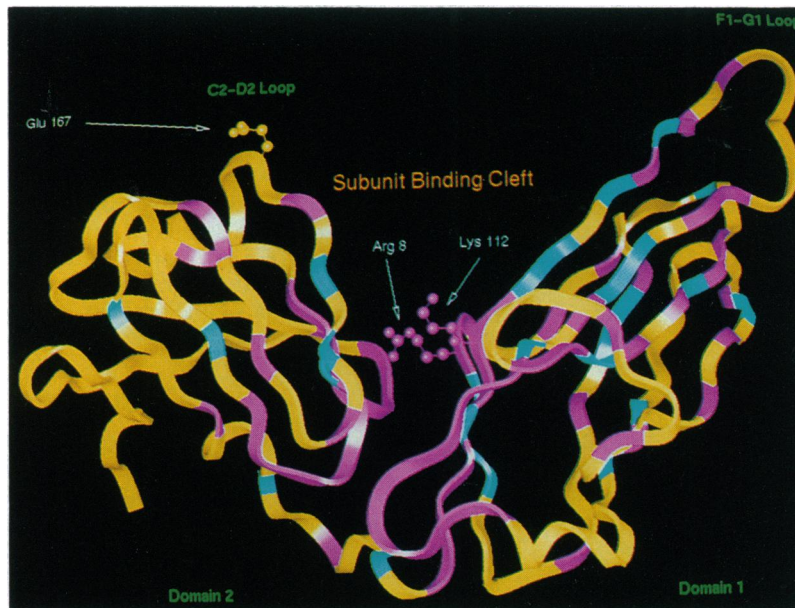


FIG. 2. Ribbon model of the crystal structure of PapD colored to indicate the homology of this protein with FimC. Identical residues are shown in purple while conservatively substituted residues are shown in blue. The side chains of the invariant residues Arg-8 and Lys-112 and the variable residue Glu-167 are indicated.

from the periplasm. Periplasm containing FimC and FimH (Fig. 3B, lane 1) was applied to mannose-Sepharose beads. A FimC-FimH complex was eluted with D-mannose (data not shown) and with methyl  $\alpha$ -D-mannopyranoside (Fig. 3B, lane 3). Lane 4 in Fig. 3B shows that FimC alone is not retained by the mannose-Sepharose beads, but requires an interaction with FimH to be retained on the beads. We verified the identity of the eluted bands as FimC and FimH by Western blotting (data not shown) and by amino-terminal sequencing (Fig. 3 legend). Our results with the FimC-FimH complex demonstrate that FimH is folded in the complex such that the mannose-binding domain is in a native state and accessible for substrate binding. These results parallel those reported with the PapD-PapG complex (6, 8).

**Structure-Function Relationship Between PapD and FimC.** Given the predicted structural and observed biochemical relatedness between the chaperone proteins, we decided to test whether PapD could function in place of FimC in the assembly of type 1 pili. A knockout mutation in *fimC* (*fimC1*)

on pJP5 abolished the ability of ORN103/pJP5 to produce pili as analyzed by EM (data not shown) and to mediate MSHA of guinea pig erythrocytes (Table 2). Remarkably, pLS101 (*papD*) complemented the *fimC1* null mutation (Table 2). Furthermore, we have demonstrated that both ORN103/pJP5 plus pLS101 (*papD*) and ORN103/pJP5 plus pJP4 (*fimC*) produce equivalent amounts of pili (data not shown). ORN103/pJP5 plus pLS101 (*papD*), however, had an 8-fold lower MSHA titer than ORN103/pJP5 plus pJP4 (*fimC*) (Table 2).

The ability of PapD to complement the *fimC1* genetic lesion confirms that the structures of PapD and FimC are highly related. The *fimC1* lesion, however, could not be complemented when PapG was coexpressed with PapD on plasmid pJP1 (*papD*, *papG*). ORN103/pJP5 (*fimC1*) plus pJP1 was MSHA-negative (Table 2) and nonpilated as determined by EM (data not shown). PapD has been shown to bind to the PapG adhesin with a high affinity (8), which apparently is greater than the affinity that PapD has for the type 1 pilus

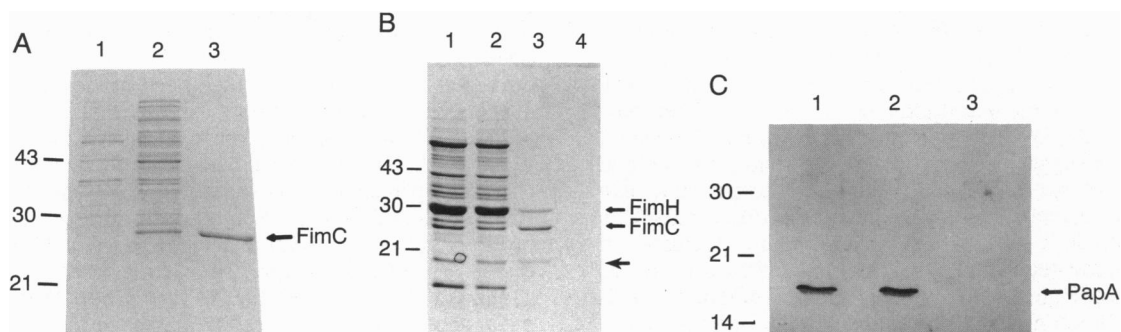


FIG. 3. Purification of FimC from the periplasm and subunit stabilization by the FimC chaperone. (A) HB101/pJP4 periplasm prepared from cells grown without (lane 1) and with (lane 2) 1 mM IPTG. A 26-kDa band, the predicted molecular mass for mature FimC, is seen only after addition of inducer. Lane 3 contains the eluate following chromatographic separation of induced periplasm as described (5). (B) Purification of the FimC-FimH complex. Lane 1 shows IPTG-induced periplasm from ORN103/pJP4 (*fimC*) plus pRT4 (*fimH*). The unbound material and the methyl  $\alpha$ -D-mannopyranoside eluate from the mannose-Sepharose beads are shown in lanes 2 and 3, respectively. Shown in lane 4 is the eluate from induced ORN103/pJP4 (*fimC*) periplasm treated identically to the material in lane 3. The amino-terminal sequence of the bands in lane 3 was obtained by verifying the identity of the eluted proteins as FimH (FA-KTAN) and FimC (—AL—ATRVIIY). The lower band in lane 3 (arrow) was identified by amino-terminal sequencing as a carboxyl-terminal truncate of FimH. (C) Western blot analysis with anti-PapA antiserum of periplasmic preparations showing the ability of FimC to stabilize the PapA subunit in the periplasm. In the absence of a chaperone, the PapA subunit is degraded in the periplasm (lane 3), whereas both PapD (lane 1) and FimC (lane 2) stabilize the subunit.

Table 2. Pilus assembly directed by a heterologous chaperone

Gene(s) induced in trans	Chaperone complementation		Test of dominance	
	Type 1* ( <i>fimC1</i> )	Pap <sup>†</sup> ( <i>papD1</i> )	Type 1* ( <i>fimC</i> <sup>+</sup> )	Pap <sup>†</sup> ( <i>papD</i> <sup>+</sup> )
None	3	0	850	256
<i>fimC</i>	850 <sup>‡</sup>	0	512	190
<i>papD</i>	100 <sup>‡</sup>	128	512	256
<i>papD, papG</i>	4	ND	768	ND
<i>papD-R8A</i>	0	0	ND	ND
<i>papD-R8G</i>	2	0	ND	ND
<i>papD-R8M</i>	0	0	ND	ND
<i>papD-E167G</i>	8	64	ND	ND
<i>papD-E167T</i>	8	96	ND	ND

Hemagglutination titer (9, 21) was used to measure pilus assembly and represents the dilution of bacteria that causes 50% hemagglutination of guinea pig erythrocytes for type 1 pili or human erythrocytes for P pili. Titers presented represent an average of at least three experiments. ND, not determined.

\*Hemagglutination sensitive to 0.5% D-mannose.

<sup>†</sup>Hemagglutination sensitive to 0.1 mM Gal( $\alpha$ 1-4)Gal.

<sup>‡</sup>Total amounts of type 1 pili and the relative ratio of FimH per milligram of FimA were measured and found to be equivalent in *papD*- and *fimC*-complemented strains.

proteins. As a consequence, coexpression of PapG with PapD apparently titrates PapD away from the type 1 pilus proteins, resulting in their misassembly. An alternative explanation for the MSHA-negative phenotype is that the PapD-PapG complex is targeted to the type 1 outer-membrane usher, FimD, but is not efficiently uncapped, resulting in a block in pilus assembly. We favor the former explanation since ORN103/pJP3 (wild-type type 1) plus pJP1 (*papD, papG*) has a wild-type MSHA-positive titer (Table 2), indicating that there is no dominant block to pilus assembly when PapD and PapG are coexpressed in a wild-type background.

**Subunit Recognition in Heterologous Systems Requires the Chaperone Cleft.** Slonim *et al.* (9) have shown that the highly conserved cleft region of PapD contains the subunit binding site with the invariant residue Arg-8 having a critical role in subunit recognition. We investigated the effect of three mutations in Arg-8 on the ability of PapD to modulate type 1 pilus assembly. Arg-8 was changed to alanine or glycine to completely remove the putative interactive Arg-8 side chain or to a methionine to maintain the bulk of the arginine side chain while removing the charge. None of these amino acid substitutions significantly affected the stability of PapD in the periplasmic space (9). All three mutations completely abolished the ability of PapD to assemble type 1 pili as well as P pili (Table 2), arguing that the recognition of both P and type 1 subunits by PapD requires the invariant Arg-8 cleft residue.

It has been suggested that the variable residues located in the loop structures that border the binding site cleft may play a role in chaperone specificity. One such residue in PapD is Glu-167, which is part of a negatively charged cluster of amino acids located in the loop that joins  $\beta$  strand C2 to D2 at the tip of domain 2 (3, 7, 9). Replacement of Glu-167 with glycine or threonine had only a modest effect on the ability of PapD to mediate P pilus assembly (Table 2) but significantly reduced the ability of PapD to direct type 1 pilus assembly, arguing for a role of this negatively charged region of the loop in interacting with type 1 subunits.

**FimC Binds PapA But Does not Assemble P Pili.** FimC was unable to complement the *papD1* lesion on pPAP37. ORN103/pPAP37 plus pJP4 (*fimC*) was hemagglutination-negative (Table 2) and nonpilated as determined by EM (data not shown). Interestingly, the inability of FimC to mediate P pilus assembly was not due to the lack of interaction with the

major subunit, PapA. A general property of P pilus subunits is that they follow nonproductive pathways that lead to aggregation and proteolytic degradation in the absence of an interaction with a periplasmic chaperone. Thus, PapA was proteolytically degraded when expressed from plasmid pPAP43 (23) in strain ORN103 (Fig. 3C, lane 3). Surprisingly, when PapA was coexpressed with either PapD or FimC, both chaperones were able to bind to PapA and stabilize the subunit in the periplasm so that it was detectable by Western blotting (Fig. 3C, lanes 1 and 2). The inability of FimC to direct the assembly of the Pap subunits into pili is presumably a reflection of the inability of FimC to orchestrate other critical interactions.

## DISCUSSION

Periplasmic chaperones are a large family of proteins that are essential in the postsecretional assembly of higher order structures (1-9). Virtually nothing is known about periplasmic trafficking and targeting of proteins to the outer membrane; however, there is evidence that import of type 1 pilin subunits into the periplasmic space is dependent on the *sec* system (25). We suggest that in Gram-negative bacteria periplasmic chaperones are a component of a secretory pathway that also includes an outer-membrane transport protein (*usher*) to which the chaperone targets cognate proteins (11). Due to the interactive nature of pilus subunit proteins, this periplasmic transport system is probably required to receive nascently translocated subunits imported by the *sec* machinery. One defined role of periplasmic chaperones in this secretory pathway is to direct nascently translocated proteins down productive biological pathways by capping interactive surfaces to prevent aggregate formation and proteolytic degradation (8).

Previous work on the PapD chaperone, required for the assembly of P pili, has implicated the conserved cleft of the molecule in subunit binding (9). The recently determined crystal structure of PapD complexed with a PapG carboxyl-terminal peptide supported this conclusion by revealing that the peptide was anchored in the cleft by the invariant Arg-8 and Lys-112 residues and that it interacted along the G1 strand of PapD and with the F1 to G1 loop (M. Kuehn, D. Ogg, J. Kilberg, L.N.S., T. Bergfors, K. Flemmer, and S.J.H., unpublished results). Our demonstration of complementation of a *fimC1* null allele by PapD and the dependence of that activity on the Arg-8 residue suggests that the conserved cleft in periplasmic chaperones serves a common function in pilus biogenesis (Table 2). On the basis of the complementation results presented here, we suggest that the positively charged invariant cleft residues form a part of a universal anchor used by members of this protein family (Table 3) in chaperone-subunit interactions.

The differential effect of substitution at Glu-167 on assembly of P pili versus type 1 pili suggests a possible role for the C2-D2 loop in chaperone-subunit interactions (Table 2). The strong effect of the Glu-167 mutations on type 1 pilus assembly may reflect an involvement of the negatively charged region of the C2-D2 loop in interacting with the type 1 pilus subunits. This promotes the intriguing hypothesis that perhaps loops at the tips of both domains (Fig. 2) are important in chaperone-subunit interactions. In this model, a subunit would be anchored in the cleft by the invariant Arg-8 residue and make interactions along the faces of both domains of the chaperone.

Although *papD* complementation of the *fimC1* null allele produced type 1 pili and caused MSHA of guinea pig erythrocytes, the effective titer of these strains was 8-fold lower than the isogenic *fimC*-complemented strain (Table 2). Surprisingly, the lower hemagglutination titer was not due to decreased amounts of FimH-containing pili, since *fimC*- and



Table 3. Thirteen members of the chaperone superfamily

Chaperone	Mass, kDa	Organism	Surface structure	Homology to PapD*, %
PapD	28.5	<i>E. coli</i>	P pilus	
FimC	26	<i>E. coli</i>	Type 1 pilus	50
SfaE	23.5	<i>E. coli</i>	S pilus	62
FaeE	26.5	<i>E. coli</i>	K88 pilus	54
MrkB	25	<i>K. pneumoniae</i>	Type 3 pilus	60
HifB	25	<i>H. influenzae</i>	Pili	58
F17D	28	<i>E. coli</i>	F17 pilus	56
FanE	23	<i>E. coli</i>	K99 pilus	59
CS3-27kDa	27	<i>E. coli</i>	CS3 pilus	48
SefB	27	<i>S. enteritidis</i>	Fimbrin	55
FimB	24	<i>B. pertussis</i>	Types 2 and 3 pili	54
Caf1M	26	<i>Y. pestis</i>	F1 envelope antigen	54
PsaB	25	<i>Y. pestis</i>	pH 6 antigen	57

The grouping of chaperones presented is based on published work (3, 4), except PsaB (from GenBank).

\*Each protein was aligned to PapD, the prototype member of the chaperone superfamily, using the program GAP (version 7; Genetics Computer Group).

*papD*-complemented bacteria produced equivalent amounts of pili containing an equivalent amount of FimH per milligram of FimA (data not shown). In addition, using high-resolution EM, we discovered that type 1 pili assembled by either PapD or FimC had an identical composite architecture consisting of a short fibrillar tip structure joined end-to-end to the pilus rod (C.H.J. and S.J.H., unpublished results). Type 1 tip fibrillae are shorter than the previously described tip fibrillae of P pili (18, 19) but probably serve a similar function in presentation of the adhesin (2, 18, 19). Presumably, FimH was presented slightly differently in the PapD-assembled type 1 pili, which accounts for the lower hemagglutination titers, but the details of this "misincorporation" are not yet known.

We have shown that FimC is capable of binding to PapA in the periplasm (Fig. 3C) but fails to direct the assembly of adhesive P pili (Table 2). One explanation for these findings is that the FimC chaperone fails to interact with PapF and PapK. PapF and PapK are two specialized subunits required to initiate the assembly of the tip fibrillum and pilus rod and to join each structural element within the pilus (18, 19). A *papF*, *papK* double mutant abolishes the ability of the bacteria to produce pili (19). Although FimC binds to PapA, it may be unable to bind to other Pap subunit proteins; we have shown that FimC, although it interacts with PapG, fails to form a FimC-PapG complex that is competent to bind to receptor (data not shown). Alternatively, the FimC-subunit complexes may fail to interact with PapC, the outer membrane usher in the P pilus system (11). As with the entire chaperone family, PapD and FimC diverge considerably in the loop regions and as in domain 2 of the molecule (Figs. 1 and 2). These regions might define the specificity of the interaction of the chaperone-subunit complex with the usher.

Our studies on the function of two chaperones in pilus biogenesis allow for the formulation of a model that describes the interaction of the chaperone with subunit proteins. The subunits probably anchor to the invariant Arg-8 residue deep

in the chaperone cleft and interact along both cleft faces of domain 1 and domain 2 including the variable loops at the tips of each domain. The ability to understand the fine molecular details of the mechanism of action of molecular chaperones in the development of pilus organelles will continue to unveil general principles of macromolecular assembly. Crystals of FimC that diffract to 3.2 Å have already been generated from the purified FimC obtained from these studies, and the three-dimensional structure is currently being solved (S. Knight, personal communication).

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