EMBO reports

scientific report

Molecular mechanism of P pilus termination in uropathogenic *Escherichia coli*

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P pili are important adhesive fibres that are assembled by the conserved chaperone–usher pathway. During pilus assembly, the subunits are incorporated into the growing fibre by the donor-strand exchange mechanism, whereby the β-strand of the chaperone, which complements the incomplete immunoglobulin fold of each subunit, is displaced by the amino-terminal extension of an incoming subunit in a zip-in-zip-out exchange process that is initiated at the P5 pocket, an exposed hydrophobic pocket in the groove of the subunit. *In vivo*, termination of P pilus growth requires a specialized subunit, PapH. Here, we show that PapH is incorporated at the base of the growing pilus, where it is unable to undergo donor-strand exchange. This inability is not due to a stronger PapD–PapH interaction, but to a lack of a P5 initiator pocket in the PapH structure, suggesting that PapH terminates pilus growth because it is lacking the initiation point by which donor-strand exchange proceeds.

Keywords: donor-strand exchange; PapH; pili

EMBO reports (2006) 7, 1228–1232. doi:10.1038/sj.embor.7400833

INTRODUCTION

Gram-negative bacteria assemble adhesive fibres that mediate the initial step of various diseases (Thanassi *et al*, 1998a). The prototypical P pili are specifically required for uropathogenic *Escherichia coli* to bind to Gal-α(1–4)-Gal moieties in human kidney cells (Hoschutzky *et al*, 1989; Roberts *et al*, 1994; Dodson *et al*, 2001) and cause pyelonephritis (Roberts *et al*, 1994). P pili are encoded by the *pap* gene cluster and are assembled by means of the highly conserved chaperone–usher pathway, involving a periplasmic immunoglobulin-like chaperone and an outer membrane usher (Thanassi *et al*, 1998b; Soto & Hultgren, 1999). Six structural subunits make up a composite pilin fibre with a short-tip fibrillum joined to a rigid helical rod (Gong & Makowski, 1992). The crystal structure of the pilin protein PapK in complex with the chaperone PapD showed that the single domain of PapK adopts an immunoglobulin-like fold but lacks the seventh, carboxy-terminal G β-strand, producing a large hydrophobic groove on the side of the protein (Sauer *et al*, 1999). In a process termed 'donor-strand complementation', strand G of the amino-terminal domain of the chaperone (β-strand G_1) inserts a conserved motif of four alternating hydrophobic residues (termed 'P1–P4 residues') into four binding pockets in the hydrophobic groove of the pilus subunits (termed 'P1–P4 binding pockets'). The G_1 strand makes main-chain/main-chain hydrogen-bonding interactions with strand F, and thus provides *in trans* the β-strand that is lacking in the pilus subunit, thereby completing its immunoglobulin fold (Choudhury *et al*, 1999; Sauer *et al*, 1999).

Pilus subunits are assembled in a hierarchical manner at the usher PapC, which is a large β-barrel protein that acts as a pore across the outer membrane (Henderson *et al*, 2004). The assembly involves the non-covalent polymerization of pilin subunits by a process called 'donor-strand exchange' (DSE). All of the pilin subunits, apart from adhesin, have an extra N-terminal sequence that extends away from the subunit structure in a disordered state. This sequence, referred to as the N-terminal extension (Nte), comprises a highly conserved array of alternating hydrophobic residues, termed 'P2–P5 residues' (Soto *et al*, 1998; Sauer *et al*, 1999). As chaperone–subunit complexes are differentially targeted to the usher (Dodson *et al*, 1993; Saulino *et al*, 1998), each subunit donates its Nte to complete the immunoglobulin fold of the preceding subunit, inserting its P2–P5 residues into the corresponding P2–P5 binding pockets in the groove of the pilus subunit (Jacob-Dubuisson *et al*, 1994; Sauer *et al*, 2002; Zavialov *et al*, 2003; Vetsch *et al*, 2006). Recently, a detailed mechanism was established for DSE both *in vitro* and *in vivo*. By using X-ray crystallography and real-time electrospray ionization-mass spectrometry, it was shown that DSE involves the formation of a transient ternary chaperone–subunit–subunit complex (Remaut *et al*, 2006). Ternary complex formation is critically dependent on the interaction of the P5 pocket of the subunit with the P5 residue of the Nte of the incoming subunit. After the ternary complex is

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Received 30 June 2006; revised 1 September 2006; accepted 5 September 2006; published online 3 November 2006

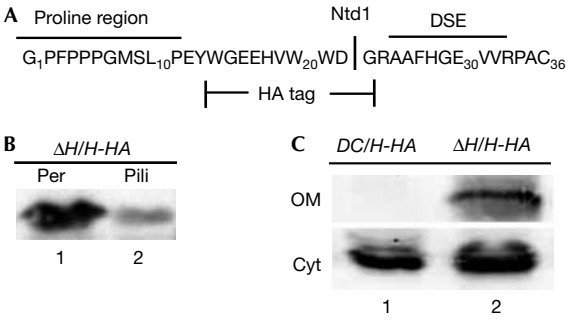

Fig 1 | PapH localizes to the outer membrane and pilus base. (**A**) The N-terminal extension (Nte) of PapH consists of Gly 1 through Cys 36. Deleting residues Pro 2–Asp 22 produced the N-terminal deleted PapH ($PapH_{Ntd1}$). A haemagglutinin (HA) epitope tag was inserted, replacing residues 14–22 (H-HA). (**B**,**C**) Western blots developed with anti-HA antiserum. (**B**) PapH-HA in periplasm extract (Per, lane 1) and purified pili (Pili; lane 2) when expressed with Δ*papH pap* operon. (**C**) PapH-HA in outer membrane (OM) and cytoplasm/periplasm (Cyt) fractions when expressed with PapD and PapC only (*DC/H-HA*; lane 1) or with Δ*papH pap* operon (Δ*H/H-HA*; lane 2). DSE, donor-strand exchange; Ntd1, N-terminal deleted.

formed, the DSE reaction proceeds through a β-strand displacement (or zip-in-zip-out) mechanism by which the P4, P3, P2 and P1 pockets are progressively occupied as the strand of the chaperone is displaced.

PapH has the ability to terminate pilus assembly (Baga *et al*, 1987). An in-frame deletion in the *papH* gene produces P pili that are very long and free from the cell (Baga *et al*, 1987), whereas overexpression of *papH* causes P pili that are short and bound to the cell. This has produced a model by which incorporation of PapH into the growing pilus both terminates growth and anchors the pilus to the cell membrane, making PapH the last subunit to be incorporated into the fibre. The molecular basis for the ability of PapH to terminate pilus assembly is however unclear. In this report, we investigate the molecular basis of pilus termination by PapH.

RESULTS AND DISCUSSION

Incorporation and localization of PapH in the pilus

PapH is similar to other pilus subunits in sequence (Normark *et al*, 1986; Baga *et al*, 1987) but differs by having a longer Nte (Fig 1A). The first 36 amino acids of PapH comprise its Nte and consist of two regions—a proline-rich region and a motif of alternating hydrophobic residues (Ala 25, Phe 27, Gly 29, Val 31), presumably responsible for participating in DSE. The proline-rich region has been suggested to be important for membrane association (Baga *et al*, 1987); however, a number of other mechanisms could be predicted, as it is unknown whether PapH is localized to the outer membrane or is even incorporated into the pilus structure.

Thus, we first set out to localize the function of PapH. We started by deleting the entire proline-rich region (Nte1) of PapH, producing the N-terminal deleted PapH ($PapH_{Ntd1}$; Fig 1A). This resulted in P pili that had similar length and distribution as in wild-type cells (supplementary Fig 1 online). The proline-rich region is therefore not responsible for the anchoring or truncation abilities of PapH. As there was no disruption in function, we chose to replace a portion of Nte1 with a haemagglutinin (HA) epitope tag (PapH-HA; Fig 1A). Bacterial cells expressing a Δ*papH pap* operon were complemented with a plasmid expressing *papH-HA* with the chaperone *papD* (pDH-HA). Negative-stain electron microscopy showed good complementation by the PapH-HA construct, with the cells expressing pili levels similar to those in wild-type cells (supplementary Fig 1 online). PapH-HA was stable in the periplasm of these cells (Fig 1B, lane 1) and the pili purified by heat purification showed incorporation of PapH-HA into the pilus structure (Fig 1B, lane 2). If PapH were the last subunit incorporated into the growing pilus, then it should be localized to the outer membrane and dependent on the other pili subunits for this localization. To test this, bacterial cells expressing the Δ*papH pap* operon or only the usher *papC* were complemented with pDH-HA. When expressed with only the chaperone and usher, PapH-HA was expressed stably but failed to localize to the outer membrane (Fig 1C, lane 1). However, when expressed with the entire *pap* operon, PapH–HA was both stable and localized to the outer membrane (Fig 1C, lane 2). Loading controls for the outer membrane fractions were analysed and confirmed by using SDS–polyacrylamide gel electrophoresis (SDS–PAGE; data not shown). These results show that PapH is incorporated into the pilus structure, is localized to the outer membrane and that this localization is dependent on the other pilin subunits. This strengthens the model of PapH being the last subunit incorporated into the growing pilus, whereby its incorporation into the pilus base truncates and anchors pili, leaving PapH localized to the outer membrane.

PapH is unable to undergo DSE *in vitro*

To provide an explanation for the termination of pilus biogenesis on incorporation of PapH, we suggested that a mechanism exists that prevents PapH from undergoing DSE with any other subunits. To test the hypothesis, a new biochemical *in vitro* assembly assay was set up to monitor DSE. $PapD_{His}$–PapA or $PapD_{His}$–PapH complexes—in which PapD is tagged at the carboxyl terminus with a His_6 tag—were incubated with increasing amounts of peptides derived from the Nte of either PapA (Nte_{PapA}; residues Ala 1–Asp 19) or PapH ($Nte2_{PapH}$; residues Gly 23–Arg 33)—both of which encompass their respective DSE regions. Subunit–peptide product formation was monitored after overnight incubation at 24 °C by using a nickel-spin column, which retains the chaperone–subunit complex and the free chaperone. SDS–PAGE was then carried out to analyse the subunit–peptide product released in the flow-through samples. As some chaperone–subunit complexes have been shown to undergo spontaneous DSE-mediated polymerization (PapA) or nonspecific aggregation (PapH), and as these would compete with the process that we wished to monitor, non-aggregating mutants of PapA and PapH were used. To generate a non-polymerizing mutant of PapA, the Nte1 sequence preceding the DSE motif of PapA (Pro 2–Gln 8) was removed and Gly 15 was mutated to Asn ($PapA_{Ntd1G15N}$). Likewise, a stable mutant of PapH was generated by removing the Nte1 sequence ($PapH_{Ntd1}$; see text described above).

SDS–PAGE of the flow-through samples showed that, for PapA, the exchange reaction proceeded efficiently and the amount of subunit–peptide complexes released increased with the increase in Nte_{PapA} or $Nte2_{PapH}$ peptide concentrations (Fig 2, left panel). Thus, we found that PapA undergoes DSE with the Nte of either

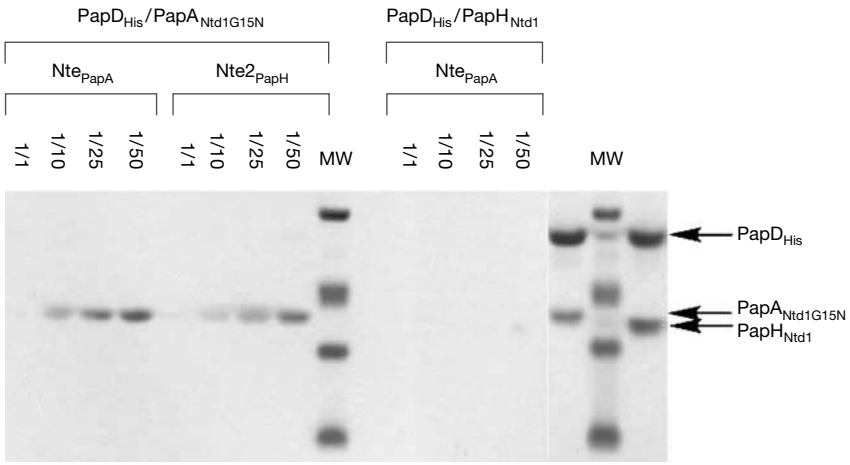

Fig 2 | *In vitro* donor-strand exchange experiments. This figure shows the results of polyacrylamide gel electrophoresis analysis of flow-through samples from the Nickel-NTA spin columns after the complexes were incubated overnight at 24 °C in the presence of Nte$_{Pap}$ peptides. Left and middle left: increasing molar ratios of 1, 10, 25 and 50 (ratio of peptide to complex) of first Nte$_{PapA}$ peptides, and second Nte2$_{PapH}$ peptides, added to the PapD$_{His}$–PapA$_{Ntd1G15N}$ complex. Middle right: increasing molar ratios of 1, 10, 25 and 50 (ratio of peptide to complex) of Nte$_{PapA}$ peptide added to the PapD$_{His}$–PapH$_{Ntd1}$ complex. Similar results were obtained by using Nte2$_{PapH}$ (not shown). MW indicates molecular weight markers (6, 14.4, 21.5 and 31.0 kDa from bottom to top). Right: the PapD$_{His}$–PapA$_{Ntd1G15N}$ complex, the molecular weight markers (MW) and the PapD$_{His}$–PapH$_{Ntd1}$ complex, with no peptide added, eluted from the spin column.

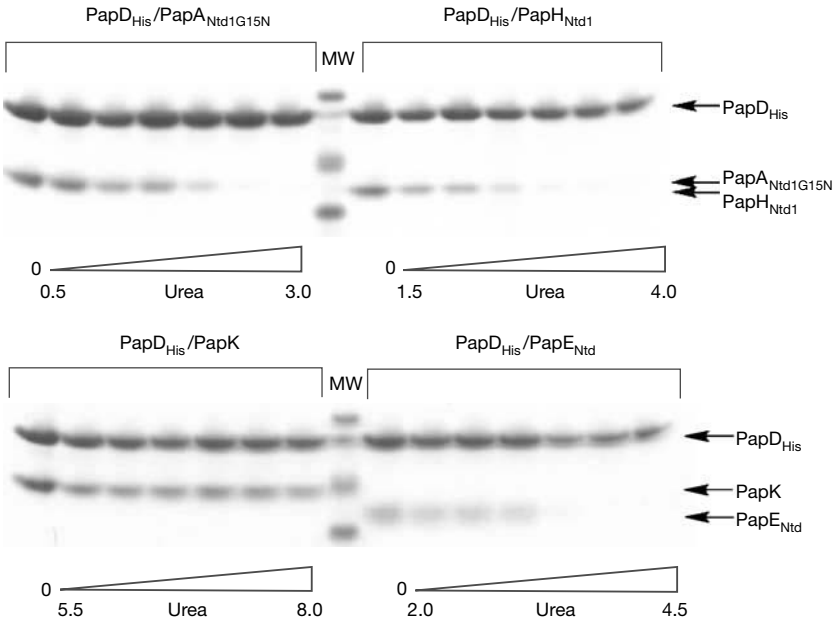

Fig 3 | Urea denaturation experiments. The various complexes indicated above the four panels were incubated with increasing amounts of urea (0–8 M); eluates, after loading onto Ni-NTA spin columns, were analysed by using polyacrylamide gel electrophoresis. Only the range that leads to complete complex dissociation is shown (except for the PapD–PapK complex, which is stable even at 8.0 M urea). MW indicates molecular weight markers (6, 14.4, 21.5 and 31.0 kDa from bottom to top).

PapA or PapH, forming Nte$_{PapA}$–PapA$_{Ntd1G15N}$ or Nte2$_{PapH}$–PapA$_{Ntd1G15N}$ products. However, the situation was very different when the PapD$_{His}$–PapH$_{Ntd1}$ complex was challenged by the Nte$_{PapA}$ or Nte2$_{PapH}$ peptides (Fig 2, right panel; only Nte$_{PapA}$ is shown, as the results are identical to those of Nte2$_{PapH}$). No exchange was detected on the gel (only trace amounts with a 50:1 ratio). Similarly, *in vitro* DSE using the full-length PapH protein yielded no product formation (results not shown). Thus, in contrast with PapA, PapH is unable to undergo DSE.

Stability of the PapD–PapH complex

One possible reason for PapH not undergoing DSE might be that the interaction between PapD and PapH is stronger compared with the other subunits, and PapD cannot be uncapped by the Nte

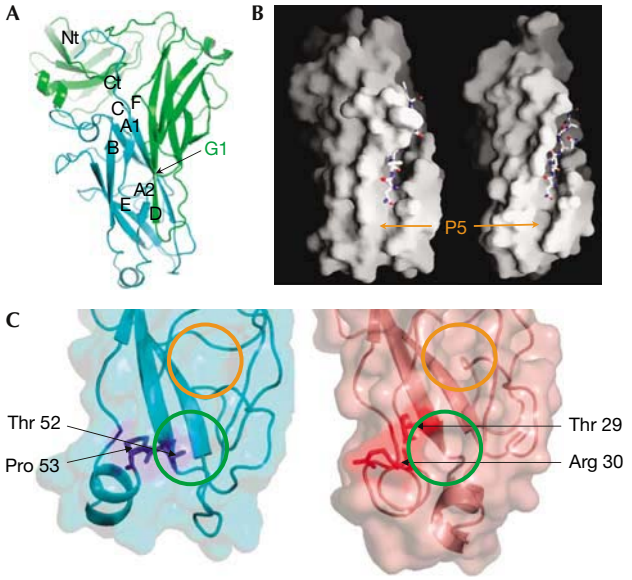

Fig 4 | Crystal structure of PapD–PapH$_{Ntd1}$ and P5 pocket of PapH and PapK. (**A**) Overall three-dimensional structure of the PapD–PapH$_{Ntd1}$ complex. PapD (green) and PapHNtd1 (blue) are shown in ribbon representations, with β-strand indicated with arrows and α-helices indicated as cylinders. Secondary structures of PapH and the G1 strand of the chaperone are labelled. (**B**) Surface representations of PapH$_{Ntd1}$ (left) and PapK (right) in complex with PapD. The position of the P5 pocket is indicated. For clarity, only residues in the G1 strand of the chaperone are shown (in a stick representation, colour-coded in red, blue and white for oxygen, nitrogen and carbon atoms, respectively). (**C**) Details of the P5 region of PapH (cyan) and the P5 pocket of PapK (red). Residues Thr 52 and Pro 53 of PapH are indicated in a stick representation, as are residues 29 and 30 of PapK. The locations of the P4 and P5 pockets in both proteins are indicated by an orange and a green circle, respectively.

of any subunit when in complex with PapH. However, affinities between chaperone and subunits cannot be measured, as subunits are not stable on their own. Instead, the strength of the chaperone–subunit interaction is typically investigated by using urea (Barnhart *et al*, 2000). For these urea denaturation experiments, various subunit–chaperone complexes were incubated with increasing amounts of urea (0–8 M at 0.5 M intervals at pH 7.5) and analysed. The results (Fig 3) show that the PapD–PapH interaction is comparable with other subunits, except PapK which is stable even at 8 M urea. Thus, the inability of PapH to undergo DSE is not due to a stronger interaction with PapD.

PapD–PapH$_{Ntd1}$ structure shows obstructed P5 pocket

PapH is the only pilin that does not undergo DSE, but shown earlier, its inability to undergo DSE is not due to an inherently more stable chaperone–subunit complex. Therefore, could PapH lack a hydrophobic groove or have crucial elements that would prevent DSE? To address this question, we determined the structure of the PapD–PapH$_{Ntd1}$ complex. Fig 4A is an overall representation of the structure of PapD–PapH$_{Ntd1}$, which is very similar to the already known structures of PapD–PapK or PapD–PapE$_{Ntd}$ (Sauer *et al*, 1999, 2002). Similar to PapK and PapE, PapH lacks the G strand of its immunoglobulin fold and PapD complements this by donating its G1 strand. The groove at position P1–P4 is very similar to that of PapK and PapE (Fig 4–C). However, PapK and PapE have a clearly defined P5 pocket, whereas PapH has none (Fig 4B). In PapH, the P5 pocket is blocked by residue Thr 52 (Fig 4C). Although conserved in PapK (Thr 29; Fig 4C), Thr 52 obstructs the P5 pocket of PapH because it is forced into the observed conformation by a kink caused by Pro 53.

The mechanism of DSE was recently examined in greater molecular detail and showed that the P5 pocket in the groove of assembling subunits has a crucial role in initiating DSE (Remaut *et al*, 2006). Using the Saf pilus system of *Salmonella enterica*, Remaut *et al* showed that (i) DSE is initiated by the interaction of the P5 residue on the Nte of the incoming subunit with the P5 pocket of the subunit that is already assembled, and (ii) obstructing the P5 pocket or mutating the P5 residues inhibits this interaction, leading to a marked decrease in ternary complex formation and a resulting inhibition of the DSE reaction. Thus, PapH does not undergo DSE, probably because it is lacking a P5 pocket. We propose that the lack of a P5 pocket explains the mechanism of PapH as a regulator of pilus length.

CONCLUSION

Using various techniques, the molecular basis by which a seemingly normal pilin subunit has such a marked role in controlling pilus length was investigated. This study shows that PapH acquires the same C-terminally truncated immunoglobulin-like fold as other pilin subunits and relies on the PapD chaperone for donor-strand complementation. PapH is then localized to the outer membrane where it is incorporated into the pilus structure, most probably by donating its Nte to complete the fold of PapA. However, we show that PapH is unable to undergo DSE. This failure was attributed to the lack of a P5 pocket in the groove of PapH. Indeed, a previous study has identified the P5 pocket as the initiator point for DSE (Remaut *et al*, 2006). Thus, the P5 residue of the Nte of PapH is able to insert into the P5 pocket of the groove of PapA, whereas no similar residue in any Nte can insert into the groove of PapH. Indeed, the absence of the P5 pocket in PapH is probably the single most important reason for its role as a terminator subunit. This study firmly establishes that PapH functions through a conserved assembly mechanism in pilus biogenesis. Most pilin subunits use their P5 pocket to promote assembly into the pilus structure through DSE, whereas PapH blocks this important pocket to truncate pilus assembly and regulate pilus length. This study emphasizes the P5 pocket as a conserved assembly mechanism of pilus biogenesis in Gram-negative pathogens, which, as shown by PapH, can provide a target for disrupting the assembly of these virulence factors.

METHODS

Protein expression and purification. For details, see the supplementary information online.

PapH localization. Bacterial cells expressing pDH-HA in a Δ*papH pap* background (pEMM13) or in the presence of *papC* (pYML113) were scraped from tryptic soy agar plates (grown at 37 °C for 36 h with 0.1 μM isopropyl-β-D-thiogalactoside) and suspended in 5 mM Tris and 75 mM NaCl (pH 8.0). Resuspended cells were heated at 65 °C to remove bound pili (Hoschutzky *et al*, 1989), and the supernatants containing pili were salt precipitated

(Kuehn *et al*, 1992). The outer membranes of the cells were purified by using ultracentrifugation and made soluble in 1% Elugent and 20 mM HEPES (pH 7.5; Saulino *et al*, 1998). Purified pili, outer membrane and cytoplasm/periplasm samples were heated in SDS buffer and 4 M urea at 95 °C, and analysed by SDS–PAGE followed by immunoblotting with a monoclonal HA antibody (Covance Inc., Berkeley, CA, USA) as the primary antibody and alkaline phosphatase-conjugated goat anti-mouse IgG (Sigma, St Louis, MO, USA) as the secondary antibody.

***In vitro* donor-strand exchange experiments.** For each DSE experiment, 50 μg of the complex ($PapD_{His}$–$PapA_{Ntd1G15N}$ or $PapD_{His}$–$PapH_{Ntd1}$) was mixed with the Nte_{PapA} or $Nte2_{PapH}$ peptides in a peptide to complex molar ratio of 0 (control), 1, 10, 25 and 50 in 50 mM Tris–HCl (pH 7.5) and 50 mM NaCl (buffer A). The mixtures were then incubated overnight at 24 °C in a shaker (150 r.p.m.). After incubation, 100 μl (50 μg) of the mixture was loaded onto the Nickel-NTA spin columns that were prewashed with buffer A. The columns were then centrifuged for 2 min at 700*g* and the flow-through samples re-loaded two more times. The flow-through samples were then analysed by using SDS–PAGE.

Urea denaturation experiments. For each urea experiment, 50 μg of $PapD_{His}$–$PapA_{Ntd1G15N}$, $PapD_{His}$–$PapH_{Ntd1}$, $PapD_{His}$–PapK and $PapD_{His}$–$PapE_{Ntd}$ was incubated in urea solutions ranging from 0 to 8.0 M in 50 mM Tris–HCl (pH 7.5) and 300 mM NaCl (buffer A) for 15 min at 24 °C. A control mixture of each complex without urea was also prepared. A 100 μl portion of the preincubated urea–protein (50 μg of complex) mixture was then loaded on pre-equilibrated Nickel-NTA spin columns and centrifuged for 2 min at 700*g*. The columns were washed with 300 μl of the corresponding urea solution. The urea was removed by adding 300 μl of buffer A. The remaining complexes were eluted with a buffer containing 250 mM imidazole (buffer B) and analysed by using SDS–PAGE.

Crystallization and structure determination. For details, see the supplementary information online. Coordinates of the PapD–$PapH_{Ntd1}$ complex have been deposited to the Protein Data Bank (entry code 2j2z).

Supplementary information is available at *EMBO reports* online (http://www.emboreports.org).

ACKNOWLEDGEMENTS

This work was supported by grants from the Medical Research Council (to G.W.; no. 58149) and the National Institutes of Health (to S.H.).

REFERENCES

Baga M, Norgren M, Normark S (1987) Biogenesis of *E. coli* Pap pili: papH, a minor pilin subunit involved in cell anchoring and length modulation. *Cell* **49:** 241–251

Barnhart MM, Pinkner JS, Soto GE, Sauer FG, Langermann S, Waksman G, Frieden C, Hultgren SJ (2000) PapD-like chaperones provide the missing information for folding of pilin proteins. *Proc Natl Acad Sci USA* **97:** 7709–7714

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

Dodson KW, Jacob-Dubuisson F, Striker RT, Hultgren SJ (1993) Outer-membrane PapC molecular usher discriminately recognizes periplasmic chaperone–pilus subunit complexes. *Proc Natl Acad Sci USA* **90:** 3670–3674

Dodson KW, Pinkner JS, Rose T, Magnusson G, Hultgren SJ, Waksman G (2001) Structural basis of the interaction of the pyelonephritic *E. coli* adhesin to its human kidney receptor. *Cell* **105:** 733–743

Gong M, Makowski L (1992) Helical structure of P pili from *Escherichia coli*. Evidence from X-ray fiber diffraction and scanning transmission electron microscopy. *J Mol Biol* **228:** 735–742

Henderson NS, So SS, Martin C, Kulkarni R, Thanassi DG (2004) Topology of the outer membrane usher PapC determined by site-directed fluorescence labeling. *J Biol Chem* **279:** 53747–53754

Hoschutzky H, Lottspeich F, Jann K (1989) Isolation and characterization of the α-galactosyl-1,4-β-galactosyl-specific adhesin (P adhesin) from fimbriated *Escherichia coli*. *Infect Immun* **57:** 76–81

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

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

Normark S, Lindquist S, Lindberg F (1986) Chromosomal β-lactam resistance in enterobacteria. *Scand J Infect Dis Suppl* **49:** 38–45

Remaut H, Rose RJ, Hannan TJ, Hultgren SJ, Radford SE, Ashcroft AE, Waksman G (2006) Donor-strand exchange in chaperone-assisted pilus assembly proceeds through a concerted β strand displacement mechanism. *Mol Cell* **22:** 831–842

Roberts JA, Marklund BI, Ilver D, Haslam D, Kaack MB, Baskin G, Louis M, Mollby R, Winberg J, Normark S (1994) The Gal(α1–4)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* **91:** 11889–11893

Sauer FG, Futterer K, Pinkner JS, Dodson KW, Hultgren SJ, Waksman G (1999) Structural basis of chaperone function and pilus biogenesis. *Science* **285:** 1058–1061

Sauer FG, Pinkner JS, Waksman G, Hultgren SJ (2002) Chaperone priming of pilus subunits facilitates a topological transition that drives fiber formation. *Cell* **111:** 543–551

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

Soto GE, Hultgren SJ (1999) Bacterial adhesins: common themes and variations in architecture and assembly. *J Bacteriol* **181:** 1059–1071

Soto GE, Dodson KW, Ogg D, Liu C, Heuser J, Knight S, Kihlberg J, Jones CH, Hultgren SJ (1998) Periplasmic chaperone recognition motif of subunits mediates quaternary interactions in the pilus. *EMBO J* **17:** 6155–6167

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

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

Vetsch M, Erilov D, Moliere N, Nishiyama M, Ignatov O, Glockshuber R (2006) Mechanism of fibre assembly through the chaperone–usher pathway. *EMBO Rep* **7:** 734–738

Zavialov AV, Berglund J, Pudney AF, Fooks LJ, Ibrahim TM, MacIntyre S, Knight SD (2003) Structure and biogenesis of the capsular F1 antigen from *Yersinia pestis*: preserved folding energy drives fiber formation. *Cell* **113:** 587–596