The unfolding of the P pili quaternary structure by stretching is reversible, not plastic

Erik Fällman¹⁺, Staffan Schedin², Jana Jass³, Bernt-Eric Uhlin⁴ & Ove Axner^{1,++}

¹Department of Physics, and ²Department of Applied Physics and Electronics, Umeå University, Umeå, Sweden,

³Department of Microbiology and Immunology, The Lawson Health Research Institute, University of Western Ontario, London, Ontario, Canada, and ⁴Department of Molecular Biology, Umeå University, Umeå, Sweden

P pili are protein filaments expressed by uropathogenic *Escherichia coli* that mediate binding to glycolipids on epithelial cell surfaces, which is a prerequisite for bacterial infection. When a bacterium, attached to a cell surface, is exposed to external forces, the pili, which are composed of $\sim 10^3$ PapA protein subunits arranged in a helical conformation, can elongate by unfolding to a linear conformation. This property is considered important for the ability of a bacterium to withstand shear forces caused by urine flow. It has hitherto been assumed that this elongation is plastic, thus constituting a permanent conformational deformation. We demonstrate, using optical tweezers, that this is not the case; the unfolding of the helical structure to a linear conformation is fully reversible. It is surmised that this reversibility helps the bacteria regain close contact to the host cells after exposure to significant shear forces, which is believed to facilitate their colonization.

Keywords: folding; helical structure; optical tweezers; PapA; uropathogenic *Escherichia coli*

EMBO reports (2005) 6, 52–56. doi:10.1038/sj.embor.7400310

INTRODUCTION

Uropathogenic *Escherichia coli* are implicated in 75%–80% of uncomplicated urinary tract infections and in severe pyelonephritis (Hultgren *et al*, 1993). Such bacteria constitute a model system for how Gram-negative bacteria in general attach to host tissue by pili structures on their surface (Sauer *et al*, 2000b). However, it has remained largely unknown as to how the physical properties of pili influence the ability of a bacterium to withstand

shear forces, such as those from the rinsing action of urine. Bacterial binding is commonly mediated by multiple pili. To understand the interactions from multi-pili binding, one needs to assess the physical properties of an individual pilus. Such measurements have hitherto been nontrivial to perform. However, by access to modern techniques for force measurements in biological systems, and in particular optical tweezers (Fällman *et al*, 2004), important properties of individual pili, such as their force-versus-elongation response, can now be assessed. Examples of entities that have been studied by such techniques are the adhesion forces of type 1 pili (Liang *et al*, 2000), the twitching motility of *Neisseria gonorrhoeae* mediated by type IV pili retraction (Merz *et al*, 2000) and elongation properties of P pili (Jass *et al*, 2004).

As illustrated in Fig 1A, the main part of a P pilus, which is expressed predominantly by isolates from the upper urinary tract (Johnson & Russo, 2002; Russo & Johnson, 2003), constitutes a thin micron-long helical rod (Bullitt *et al*, 1992; Sauer *et al*, 2000a), composed of $\sim 10^3$ PapA subunits of molecular mass 16.5 kDa, coupled to their nearest neighbours by a β -strand complementation (Choudhury *et al*, 1999; Sauer *et al*, 1999, 2002; Zavialov *et al*, 2003) and combined in a right-handed helical arrangement with 3.28 subunits per turn (Gong & Makowski, 1992; Jacob-Dubuisson *et al*, 1993; Bullitt & Makowski, 1995; Bullitt *et al*, 1996). Most of the elongation properties of a P pilus are therefore governed by the PapA rod. Recent studies have shown that a single P pilus elongates by a passage through three elongation regions when exposed to mechanical stress (Jass *et al*, 2004). Region I (for elongations of up to $\sim 20\%$ of its relaxed length; Bullitt & Makowski, 1995; Jass *et al*, 2004) is characterized by a linear force-versus-elongation response and is considered to originate from an elastic stretching of the quaternary structure of the PapA rod (Jass *et al*, 2004; Fig 1B). Region II (for elongation up to a few times its relaxed length; Bullitt & Makowski, 1995; Jass *et al*, 2004) is characterized by an elongation under constant force and originates from the unfolding of the helical rod (Jass *et al*, 2004; Fig 1C). Region III, finally, is characterized by a nonlinear force-versus-elongation response and corresponds to a linearization of the head-to-tail interaction between the subunits (Jass *et al*, 2004; Fig 1D).

¹Department of Physics, and ²Department of Applied Physics and Electronics, Umeå University, 901 87 Umeå, Sweden

³Department of Microbiology and Immunology, The Lawson Health Research Institute, University of Western Ontario, 268 Grosvenor Street, London, Ontario N6A 4V2, Canada

⁴Department of Molecular Biology, Umeå University, 901 87 Umeå, Sweden

*Corresponding author. Tel: +46 90 786 6775; Fax: +46 90 786 6673;

E-mail: erik.fallman@physics.umu.se

++Corresponding author. Tel: +46 90 786 6754; Fax: +46 90 786 6673;

E-mail: ove.axner@physics.umu.se

Fig 1 | Schematic illustration of a P pilus in its unstretched native form (A) as well as in three different elongation regions (I-III; B-D). A P pilus consists of a PapA rod (6.8 nm in diameter) arranged in a helix (Gong & Makowski, 1992) that is anchored to a bacterial membrane by PapH (Båga *et al*, 1987) and connected to a fibrillum by a PapK adaptor (Jacob-Dubuisson *et al*, 1993). The fibrillum tip (15 nm long and 2-3 nm in diameter; Bullitt & Makowski, 1998) is a flexible structure composed of PapE (Lindberg *et al*, 1986), PapF (Lindberg *et al*, 1986) and the PapG (Lindberg *et al*, 1987; Kuehn *et al*, 1992; Jacob-Dubuisson *et al*, 1993) adhesin. The forces from the adjacent-turn and the head-to-tail interactions are indicated.

The ability of a uropathogenic *E. coli* to resist the rinsing action of urine flow and to subsequently colonize tissue depends, however, not only on the static or elongation properties of the pili but also on their ability to contract. We have therefore, in this work, investigated and assessed the contraction properties of P pili.

RESULTS

A typical force-versus-elongation response from a piliated *E. coli* HB101/pPAP5 bacterium that binds to a surface, measured by optical tweezers, is shown in the supplementary information online (including supplementary Fig S1 online). On the basis of this knowledge, and in particular of the elongation-independent unfolding force of the helix of a single P pilus, F_{uf} , of 27 ± 2 pN (Jass *et al*, 2004), it is possible to investigate the contraction properties of a single P pilus under controlled conditions.

A typical elongation and contraction study of the binding mediated by P pili is shown in Fig 2. Fig 2A and 2B, respectively, represents two consecutive cycles of elongation and contraction of pili from the same bacterium. Bacterium-to-bead distances of some specific features are indicated.

Fig 2 | Elongation and contraction study of P pili. Two consecutive elongation and contraction cycles are shown, displayed by the blue and black curves in (A) and by the red and green curves in (B), respectively. The numbers in the graphs indicate important bacterium-to-bead distances. The regions of the remaining pili during contraction are indicated (I, II or III). The unfolding and folding forces are indicated with arrows.

The first elongation cycle (blue curve in Fig 2A) gave rise to, for the first 0.1 μm , a linear increase of the binding force with distance, up to ~ 170 pN, originating from a number of pili either loosely bound to the bead or mediating the force by a short pili length (either because the pili are short or because the pili mediate the force by only a fraction of their length), all elongated in their linear region. The successive roll-off (between 0.1 and 0.3 μm) originated from both a nonlinearity of the detection of the position of the bead in the trap and pili that sequentially entered their unfolding regions. At 0.3 and 0.4 μm , a number of pili detached from the bead, showing up as sudden decreases in force (from 200 to 100 pN) together with an instantaneous elongation of the bond.

The increase of the force that took place for bacterium-to-bead lengths between 0.85 and 1.05 μm was caused by one or a few short pili entering their third, nonlinear elongation region. At

1.05 μm , these pili detached, which brought the force down to ~ 50 pN and the bacterium-to-bead distance up to 1.4 μm .

The binding was, after this detachment, mediated solely by pili being in their unfolding region, as indicated by the elongation-independent force response. On the basis of the force required for unfolding of the helix of a single P pilus, F_{uf} (Jass *et al*, 2004), it could be concluded that this binding was mediated by two pili. The simultaneous unfolding of two pili continued until the bacterium-to-bead distance was 2.37 μm , where one of the two pili detached, resulting in a binding mediated by only one pilus (at a force level of ~ 27 pN).

To investigate the contraction properties of an individual pilus in its unfolding region, the elongation of the binding was stopped and reversed as soon as just one pilus mediated the binding, but before it entered its third elongation region (at 2.55 μm). If the unfolding of the helical structure of the PapA rod was plastic (irreversible), as previously suggested in the literature (Bullitt & Makowski, 1995), the contraction force would have decreased well below the level of the unfolding force of a single pilus as the bacterium-to-bead distance was decreased. The black curve in Fig 2A shows, however, that the force was maintained at the same level during contraction as during extension, at ~ 27 pN. This evidence indicates that the unfolding of the quaternary, helical structure of the PapA rod in P pili is reversible and thus not plastic.

Fig 2A further shows that the contraction of the single pilus continued at a virtually constant force down to a bacterium-to-bead distance of about 0.47 μm . At this point, the PapA rod had completely regained its helical conformation, and the pilus returned to its first elongation region. This shows that the pilus contraction is reversible over the entire region II.

Moreover, the force decreased linearly with contraction through region I until it reached the zero force level at a bacterium-to-bead distance of 0.40 μm . This provides evidence that the contraction in the linear region is also fully elastic (and thus also fully reversible).

To investigate the reproducibility of P pili binding, as well as contraction behaviour in region III, a second elongation-and-contraction cycle was performed with the same bacterium. It was found that the second elongation-and-contraction cycle, displayed by the red and green curves in Fig 2B, significantly resembles the first one. The fact that the first parts of the two force-elongation curves largely resemble each other indicates that pili bind in a similar manner for repeated interactions at the same position of the bacterium. The remaining parts of the binding behaviour also have a high degree of similarity between the two elongation cycles.

This time, the elongation of the binding was pursued until the remaining pilus was well into its third elongation region, in which the helix had fully unfolded and extended to an elongated linear conformation (which it entered at ~ 2.6 μm). The elongation was halted at a bacterium-to-bead distance of 3.47 μm , before the remaining pilus detached.

The distance between the bead and the bacterium was finally decreased a second time to investigate the contraction behaviour of the pilus in its third elongation region as well as the reproducibility of the entire contracting process. The contraction of the pilus, which fully retraced the elongation in the third region, indicates that stretching in this region is elastic, and thereby fully reversible.

However, when the pilus had been elongated into its third region, the force-versus-distance response shows hysteresis when the pilus returned from region III to region II during contraction. The pilus acted with a lower contraction force (~ 12 pN) for a short distance (for bacterium-to-bead distances from 2.7 to 2.5 μm) before the contraction force again returned to its normal value (~ 27 pN). For further contraction, the PapA rod continued to fold at a contraction force of ~ 27 pN until the entire helix was recovered (at ~ 0.47 μm). Beyond this, the pilus again contracted elastically in its region I until it had been fully relieved of its tension, in a similar way to the first contraction cycle. The remaining negative force at the end of the retraction has been seen in some measurements, and it is believed to originate from either a drift of the system during the time the measurement was performed (in this case 23 min, because of a shift of the height of the bead in the trap) or a repelling force from pili protruding from the bacterium.

The retracting behaviour shown in Fig 2, which shows an elastic response in elongation regions I and III, a reversible unfolding behaviour in region II, including a lower contraction force when the pilus returns from region III to region II, was found in all elongation-and-contraction studies (~ 100), and is therefore considered to constitute a 'typical' contraction behaviour of an elongated P pilus.

DISCUSSION

This work has shown that a PapA rod, in general, contracts (that is, refolds its helical structure) spontaneously after elongation. This implies that unfolding of the quaternary structure of the PapA rod does not constitute a permanent plastic deformation, as has been proposed (Bullitt & Makowski, 1995); instead, it represents a reversible conformation-changing elongation.

This work has also shown that the PapA rod refolds its helical structure at a force, F_r , that is, within the measurement errors, equal to the unfolding force, F_{uf} , given by 27 ± 2 pN (Jass *et al*, 2004). The contraction force of ~ 27 pN is considered to originate from a sequential 'adjacent-turn-interaction' that transforms the thin fibrillar conformation of the PapA polymer into a right-handed helical rod by connecting the first unfolded PapA subunit in the linear conformation (the n th subunit) to the $(n-3)$ th and/or $(n-4)$ th subunits in the folded part of the PapA rod (Bullitt *et al*, 1996; Soto *et al*, 1998; Fig 1C). When the n th (unfolded) subunit has been docked, the subsequent subunit (the $(n+1)$ th) will automatically be dragged closer to the folded part of the helical rod so that its complementary surface can interact with the corresponding surface(s) on the $(n-2)$ th and/or $(n-3)$ th units. This adjacent-turn-interaction folding proceeds in a serial manner until the entire PapA rod has been refolded. This conclusion is supported by the fact that the contraction is independent of elongation. These results thus suggest that the binding force between the complementary surfaces in adjacent turns of the helix is equal to the unfolding force, F_{uf} , and thus that the unfolding of the pili is fully reversible.

This sequential folding can, however, only take place when at least one turn of the rod has been formed. When the entire rod has been extended, as takes place when a pilus has been elongated into its third elongation region, there are no PapA units that are close enough for the complementary surfaces at one turn to mediate any force to the adjacent turn (Fig 1D). This implies that

the n th subunit to $(n-3)$ th and/or $(n-4)$ th subunit folding can only take place when at least some part of the rod is folded. The only force that can contract the fully extended pilus under these conditions is the 'head-to-tail interaction' between subsequent subunits, between the $(n-1)$ th and the n th subunits (Bullitt *et al*, 1996; Soto *et al*, 1998). The force corresponding to this interaction, F_{hti} , which is weaker than that between adjacent turns of the helix, can be identified in the data above as the force with which the contraction takes place when the pilus returns from region III to region II during contraction. This is shown in the lower part of the elongation-and-contraction curve in Fig 2B, which shows hysteresis, that is, for a bacterium-to-bead separation of 2.5–2.7 μm . Our measurements thereby suggest that the contraction force that corresponds to the head-to-tail interaction for P pili, F_{hti} , is ~ 12 pN.

While contraction takes place by this head-to-tail interaction, no subunits are rolled up; the contraction only takes place as a consequence of a slight decrease in binding angle between the various head-to-tail interactions linking the adjacent subunits. However, once a few subunits regain the helical conformation of one turn, a tightly coiled helix follows by the adjacent-turn-interaction in a serial manner, at a force equal to the folding force, F_f .

The 's'-shaped form of the force-versus-distance curves in region III has a certain resemblance to those from studies of other protein polymers, for example, from titin (Rief *et al*, 1997). Such an 's'-shaped form has been suggested to originate from an unravelling of the tertiary Ig-like structure domains. This is, however, not a likely explanation in the present case, as an unravelling of the tertiary Ig-like structure domains would result in the disintegration of the β -strand complementation between subunits, causing an irreversible breakdown of the PapA structure. This, in turn, would presumably not give rise to a fully reversible behaviour, as was found in our study, or even a complete breakage of the subunit–subunit complexes.

The fact that consecutive force–elongation curves have such a large degree of similarity (as can be seen from a comparison of Fig 2A,B) indicates not only that pili bind in a similar manner for repeated interactions at the same position of the bacterium, but also that the pili neither break nor get detached by a breakage of the anchorage when the force becomes large. Instead, the data suggest that pili primarily detach from the bead when the force becomes large (under the conditions prevalent in these studies).

Moreover, the fact that the PapA rod has the ability to regain its original shape quickly after exposure to forces that cause various degrees of elongation is believed to assist the bacteria in regaining close contact with the host cells even after exposure to large external forces. In turn, this is believed to facilitate their colonization and subsequent infection. It is also possible that the ability of the PapA rod to refold may constitute a target for development of new antibacterial agents.

As the elongation and contraction forces of region II of ~ 27 pN have been identified as the unfolding and folding forces of the quaternary structure of the PapA rod, respectively, it is also possible to speculate whether the folding force is the driving force when the pilus is dragged through the usher in the membrane during the biogenesis of the pilus (Jones *et al*, 1992; Thanassi *et al*, 1998).

The ability to study the unfolding–contraction mechanism of pili structures in real time has led to an improved understanding of the mechanisms behind pathogenic fibre formation–deformation and their functional features. This should aid in the development of new ways to understand, prevent and treat a variety of serious human diseases. Future studies should aim at elucidating the dynamic properties of P pili and the role of individual amino acids in the subunit–subunit interaction in the quaternary structure of the P pilus as well as other pili structures on Gram-negative bacteria.

METHODS

Bacterial strains and growth conditions. To avoid possible interference of other similar (that is, fimbrial) surface structures, the P pili were expressed in the otherwise afimbriated *E. coli* strain HB101 from the plasmid pPAP5. The *E. coli* strain HB101/pPAP5 carries the entire *pap* gene cluster on the vector pBR322 and expresses normal P pili (Lindberg *et al*, 1984). The bacteria were cultured on trypticase soy agar at 37 °C and the expression of functional pili was assessed by haemagglutination assays (Jass *et al*, 2004).

Bead-based contraction assay. Samples were prepared directly on a silanized hydrophobic coverslip by sequentially adding 25 μl of freshly resuspended *E. coli* in PBS, 5 μl of activated polystyrene beads of 9.6 μm and 5 μl of small beads in PBS (pH 7.4; Jass *et al*, 2004). Bacteria and beads were prepared according to the procedures described previously (Fällman *et al*, 2004). The mounting and measurement procedure is described in supplementary information online (including supplementary Fig S2 online).

Optical tweezers force-measuring system. Force measurements were performed by the use of an optical tweezers system, which is described in detail elsewhere (Fällman & Axner, 1997; Leitz *et al*, 2002; Fällman *et al*, 2004; Jass *et al*, 2004). The instrumentation was carefully calibrated with respect to force using two separate calibration techniques on the basis of brownian motion and Stokes drag force (Fällman *et al*, 2004). The stiffness of the optical trap was ~ 0.2 pN/nm. The velocity of the cover glass movement was 0.01 $\mu\text{m/s}$. This corresponds to a loading rate of ~ 2 pN/s.

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

ACKNOWLEDGEMENTS

This work was supported by the Swedish Research Council. Economical support for the construction of a force-measuring optical tweezers system from the Kempe Foundation is acknowledged.

REFERENCES

- Båga M, Norgren M, Normark S (1987) Biogenesis of *Escherichia coli* Pap pili—PapH, a minor pilin subunit involved in cell anchoring and length modulation. *Cell* **49**: 241–251
- Bullitt E, Makowski L (1995) Structural polymorphism of bacterial adhesion pili. *Nature* **373**: 164–167
- Bullitt E, Makowski L (1998) Bacterial adhesion pili are heterologous assemblies of similar subunits. *Biophys J* **74**: 623–632
- Bullitt E, Gong M, Makowski L (1992) A molecular bungee cord—3-dimensional helical reconstruction of bacterial adhesion pili by electron-microscopy. *Mol Biol Cell* **3**: A171
- Bullitt E, Jones CH, Striker R, Soto G, Jacob-Dubuisson F, Pinkner J, Wick MJ, Makowski L, Hultgren SJ (1996) Development of pilus organelle subassemblies *in vitro* depends on chaperone uncapping of a beta zipper. *Proc Natl Acad Sci USA* **93**: 12890–12895

- 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
- Fällman E, Axner O (1997) Design for fully steerable dual-trap optical tweezers. *Appl Opt* **36**: 2107–2113
- Fällman E, Schedin S, Jass J, Andersson M, Uhlin BE, Axner O (2004) Optical tweezers based force measurement system for quantitating binding interactions: system design and application for the study of bacterial adhesion. *Biosens Bioelectron* **19**: 1429–1437
- Gong MF, 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
- Hultgren SJ, Abraham S, Caparon M, Falk P, Stgeme JW, Normark S (1993) Pilus and nonpilus bacterial adhesins—assembly and function in cell recognition. *Cell* **73**: 887–901
- Jacob-Dubuisson F, Heuser J, Dodson K, Normark S, Hultgren S (1993) Initiation of assembly and association of the structural elements of a bacterial pilus depend on 2 specialized Tip proteins. *EMBO J* **12**: 837–847
- Jass J, Schedin S, Fällman E, Ohlsson J, Nilsson U, Uhlin BE, Axner O (2004) Physical properties of *Escherichia coli* P pili measured by optical tweezers. *Biophys J* **87** (in press). doi:10.1529/biophysj.104.044867
- Johnson JR, Russo TA (2002) Uropathogenic *Escherichia coli* as agents of diverse non-urinary tract extraintestinal infections. *J Infect Dis* **186**: 859–864
- Jones CH, Jacobdubuisson F, Dodson K, Kuehn M, Slonim L, Striker R, Hultgren SJ (1992) Adhesin presentation in bacteria requires molecular chaperones and ushers. *Infect Immun* **60**: 4445–4451
- Kuehn MJ, Heuser J, Normark S, Hultgren SJ (1992) P pili in uropathogenic *Escherichia coli* are composite fibers with distinct fibrillar adhesive tips. *Nature* **356**: 252–255
- Leitz G, Fällman E, Tuck S, Axner O (2002) Stress response in *Caenorhabditis elegans* caused by optical tweezers: wavelength, power, and time dependence. *Biophys J* **82**: 2224–2231
- Liang MN, Schmith SP, Metallo SJ, Choi IS, Prentiss M, Whitesides GM (2000) Measuring the forces involved in polyvalent adhesion of uropathogenic *Escherichia coli* to mannose-presenting surfaces. *Proc Natl Acad Sci USA* **97**: 13092–13096
- Lindberg F, Lund B, Normark S (1984) Genes of pyelonephritogenic *E. coli* required for digalactoside-specific agglutination of human cells. *EMBO J* **3**: 1167–1173
- Lindberg F, Lund B, Normark S (1986) Gene-products specifying adhesion of uropathogenic *Escherichia coli* are minor components of pili. *Proc Natl Acad Sci USA* **83**: 1891–1895
- Lindberg F, Lund B, Johansson L, Normark S (1987) Localization of the receptor-binding protein adhesin at the tip of the bacterial pilus. *Nature* **328**: 84–87
- Merz AJ, So M, Sheetz MP (2000) Pilus retraction powers bacterial twitching motility. *Nature* **407**: 98–102
- Rief M, Gautel M, Oesterhelt F, Fernandez JM, Gaub HE (1997) Reversible unfolding of individual titin immunoglobulin domains by AFM. *Science* **276**: 1109–1112
- Russo TA, Johnson JR (2003) Medical and economic impact of extraintestinal infections due to *Escherichia coli*: focus on an increasingly important endemic problem. *Microbes Infect* **5**: 449–456
- 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, Barnhart M, Choudhury D, Knights SD, Waksman G, Hultgren SJ (2000a) Chaperone-assisted pilus assembly and bacterial attachment. *Curr Opin Struct Biol* **10**: 548–556
- Sauer FG, Mulvey MA, Schilling JD, Martinez JJ, Hultgren SJ (2000b) Bacterial pili: molecular mechanisms of pathogenesis. *Curr Opin Microbiol* **3**: 65–72
- 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
- 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, Lombardo MJ, Roth R, Heuser J, Hultgren SJ (1998) The PapC usher forms an oligomeric channel: implications for pilus biogenesis across the outer membrane. *Proc Natl Acad Sci USA* **95**: 3146–3151
- 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