Nucleator function of CsgB for the assembly of adhesive surface organelles in *Escherichia coli*

Zhao Bian and Staffan Normark¹

Microbiology and Tumorbiology Center (MTC), Karolinska Institute, S-171 77 Stockholm, Sweden

¹Corresponding author
e-mail: Staffan.Normark@mtc.ki.se

Curli are surface organelles in *Escherichia coli* that assemble outside the bacterium through the precipitation of secreted soluble CsgA monomers, requiring the CsgB nucleator protein. Using immunoelectron microscopy and immunoblotting assays, CsgB is shown to be located on the bacterial surface and also as a minor component of wild-type curli. CsgB lacking its 20 N-terminal residues when fused to maltose-binding protein (MBP) can still trigger polymerization of CsgA monomers *in vivo*. However, the resulting surface organelles are only formed at one of the two bacterial poles and are morphologically distinct from wild-type curli. These Bfco organelles (CsgB-Free Curli-related Organelles) are highly regular structures reacting with anti-CsgA, but not anti-CsgB antibodies. The CsgB of the active MBP–CsgBII fusion is surface exposed but, unlike the native CsgB in wild-type curli, is not detectable in the Bfco organelles. Overexpression of *csgB* within a *csgA* mutant results in the formation of short CsgB polymers on the cell surface. It is suggested that in wild-type bacteria, both CsgA and CsgB are secreted proteins. Interaction between CsgA and CsgB triggers wild-type curli formation, resulting in CsgA–CsgB heteropolymers, while surface-anchored CsgB in MBP–CsgBII triggers morphologically distinct, CsgB-free/CsgA Bfco organelles. In the absence of CsgA, CsgB can self-assemble into polymers.

Keywords: assembly/curli organelle/immunoelectron microscopy/MBP–CsgB fusions/nucleator

Introduction

Bacterial fimbriae are filamentous appendages, ranging from 2 to 8 nm in diameter on the surface of bacteria, that mediate bacteria–bacteria and bacteria–host cell interactions through their ability to bind specific structures on the cell surfaces (Duguid *et al.*, 1955; Hultgren *et al.*, 1991). In addition to classical fimbriae, many clinical *Salmonella* and *Escherichia coli* isolates as well as certain strains of *E. coli* K12, such as MC4100, express masses of thin, irregular and highly aggregated surface fibres denoted curli, when grown below 37°C, at low osmolarity during stationary phase (Olsen *et al.*, 1989; Collinson *et al.*, 1991; Sukupolvi *et al.*, 1997). These curli fibres are morphologically distinct from the rigid Type 1 pili also formed by *E. coli* K12 (Brinton, 1965). Curliated bacteria bind a series of host proteins such as fibronectin,

laminin, plasminogen and human contact phase proteins (Olsen *et al.*, 1989; Arnqvist *et al.*, 1992; Sjöbring *et al.*, 1994; Ben Nasr *et al.*, 1996). In addition, bacterial colonies of a curli-producing strain stain with the dye Congo red. So far, six *csg* genes located in a gene cluster at 23.8 minutes have been shown to be involved in the regulation and biogenesis of curli, and the functions of some gene products are now becoming clear. The major subunit protein CsgA is a 15.3 kDa protein encoded by *csgA* (Olsen *et al.*, 1993). The co-transcribed *csgB* gene product shows high sequence homology to CsgA, with 49% similarity and 30% identity, and was therefore speculated to constitute a minor component of curli (Arnqvist *et al.*, 1994), even though it was possible to identify only CsgA in a purified curli preparation (Collinson *et al.*, 1991; Olsen *et al.*, 1993). The genes *csgD*, *csgE*, *csgF* and *csgG* are all transcribed in the opposite orientation to the *csgBA* operon, and are involved in curli formation, since polar mutations in each of them abolish the expression of curli (Hammar *et al.*, 1995). CsgD is a transcriptional activator required for the expression of *csgA* and other *csg* genes, while CsgE, CsgF and CsgG act at different levels in the subunit secretion process (our unpublished data).

The bacterial strategy to assemble fimbriae such as P and Type 1 fimbriae has been carefully studied (Hultgren *et al.*, 1991). This pathway involves *sec*-dependent transport across the cytoplasmic membrane of pilus subunit proteins. To prevent premature interactions, subunit proteins are complexed to periplasmic chaperones. Subunit–chaperone complexes thereafter interact with an outer membrane usher, resulting in a release of the chaperone and subsequent polymerization by addition of subunits to the base of the growing fibres (Hultgren *et al.*, 1991; Jones *et al.*, 1993). Curli are assembled on the bacterial cell surface following a different pathway that involves a precipitation reaction of secreted soluble CsgA (Hammar *et al.*, 1996). CsgB was found to be required for this process and was therefore thought to be located on the bacterial surface, acting as a nucleator for CsgA monomer, leading to the polymerization of curli fibres.

In this study, we identify the location and function of the CsgB protein for the assembly of wild-type curli. We show that the 110 C-terminal amino acids of CsgB fused to the C-terminus of maltose-binding protein (MBP) can substitute for the 131 amino acid native CsgB in the polymerization of soluble CsgA into surface organelles that are, however, morphologically different from wild-type curli. These so-called 'Bfco' organelles (CsgB-Free Curli-related Organelles) appeared thicker and more regular structures under the electron microscope as compared with wild-type curli. To explain the nature of this change in organelle morphology, immunoelectron microscopy was performed on wild-type and mutants overexpressing CsgB in the presence or absence of CsgA. The data suggest that

in wild-type, CsgB, like CsgA, is secreted across the outer membrane. However, CsgB, unlike CsgA, is prone to be anchored at the cell surface after translocation. When overexpressed in the absence of CsgA, CsgB was able to self-assemble into short polymers on the bacterial surface. When both native proteins are expressed, CsgB is believed to impose a conformational alteration on CsgA by protein–protein interactions, leading to the CsgA–CsgB heteropolymeric wild-type curli fibres. When CsgB is anchored in the outer membrane as a fusion with the periplasmic MBP protein, only CsgA polymerization is triggered, leading to the formation of highly regular structures, CsgB-free/CsgA Bfco organelles, at one of the bacterial poles.

Results

Surface-located CsgB is a minor component of wild-type curli and self-assembles into short polymers

Previous studies showed that CsgA was the only detectable protein in a purified preparation of curli in *Salmonella* and *E. coli* (Collinson *et al.*, 1991; Olsen *et al.*, 1993). The deduced amino acid sequence of CsgB, however, shares high homology with CsgA (Arnqvist *et al.*, 1994). We therefore asked whether wild-type curli would not be only composed of CsgA but also CsgB. The *E. coli* strains MC4100 (*csgA*⁺, *csgB*⁺), MHR263 (MC4100 *recA*, *csgB*), MHR204 (MC4100 *csgA*) and MHR222 (MC4100 *csgA*, *csgB*) were therefore grown on YESCA plates (Hammar *et al.*, 1995) under curli-proficient conditions for >48 h before performing immunoelectron microscopy and immunoblotting with the affinity-purified anti-CsgB peptide antibody ZB- α B.

As shown in Figure 1, curli expressed by MC4100 were decorated with gold particles when anti-CsgB antibody was probed (Figure 1A), suggesting that CsgB indeed is present in wild-type curli fibres. We also overexpressed CsgB by subcloning a full-length version of *csgB*, including the signal peptide, into the expression vector pKK223-3, resulting in pZB-KB1 (Table I), where *csgB* was expressed under the isopropyl- β -D-thiogalactopyranoside (IPTG)-inducible *tac* promoter. pZB-KB1 was next transformed into the *csgB*-deficient strain MHR263. Overexpression of CsgB in MHR263/pZB-KB1 resulted in an abundance of curli-like fibres on the cell surface. These fibres were labelled intensively by the gold particles when probed with anti-CsgB antibody (Figure 1B), indicating that more CsgB proteins are present in these fibres than in wild-type curli. Interestingly, gold particles frequently were located at sites where curli fibres appeared to branch (Figure 1B2–B4).

As shown in Figure 2, CsgB protein was detected by immunoblot in wild-type strain MC4100 (Figure 2, lanes 1– and 2+), but not in the negative control MHR263 (Figure 2, lane 7–). Curli fibres are highly insoluble in SDS (Collinson *et al.*, 1991; Olsen *et al.*, 1993), resulting in the presence of high oligomeric forms in SDS–PAGE stacking gels without prior treatment with 90% formic acid (Hammar *et al.*, 1996). In MC4100, an extra signal was detected by anti-CsgB antibody at the top of the stacking gel, indicating that part of CsgB was present in a polymer which, like CsgA in curli fibres, is resistant to SDS treatment during the electrophoresis (Figure 2, lane

Fig. 1. Electron micrographs of immunogold-labelled wild-type curli in MC4100 (A) and curli-like fibres in MHR263/pZB-KB1 (B). Bacteria were grown on YESCA plates with or without carbenicillin under curli-proficient conditions for >48 h. Cells were incubated with CsgB-specific antibody ZB- α B and labelled with protein A–gold particles (10 nm in diameter). Magnification: (A) $\times 60\,000$; (B1) $\times 20\,000$; (B2) and (B3) $\times 150\,000$; (B4) $\times 200\,000$.

1–). For MHR263/pZB-KB1, more SDS-insoluble material appeared at the top of the stacking gel (Figure 2, lane 3–) analysed by anti-CsgB antibody compared with that of wild-type strain MC4100 (Figure 2, lane 1–). We also treated the cells of wild-type curli-expressing strain MC4100 and CsgB-overexpressing strain MHR263/pZB-KB1 with 90% formic acid prior to SDS–PAGE. Large amounts of monomeric CsgB were found in the separating gel that reacted with ZB- α B (Figure 2, lanes 2+ and 4+) compared with the same amount of cell lysis sample without prior formic acid treatment (Figure 2, lanes 1– and 3–), indicating that CsgB subunits are incorporated into wild-type curli fibres and even more so when being overexpressed. Like the major subunit protein CsgA, the minor subunit protein CsgB requires formic acid treatment for depolymerization, arguing that CsgB is integrated into the curli fibres and not merely associated with their exterior surface.

In MHR204 lacking the major subunit CsgA and expressing wild-type levels of CsgB, the bacterial surface was stained with more gold particles per area than the surroundings (Figure 3A) when CsgB-specific antibody was used for immunolabelling, suggesting that CsgB is exposed on the bacterial cell; however no surface structures

Table I. *Escherichia coli* strains, plasmids and antibodies used in this study

Strains/plasmids/ antibodies	Genotype/features	Source/reference
Strains		
XL1-Blue	<i>recA1 endA1 gyrA96 thi hsdR17(r_k⁻m_k⁺) supE44 recA1 lac-F'[proAB lacI^q Δ(lacZ)M15Tn10(tet^r)]</i>	Bullock <i>et al.</i> (1987)
MC4100	F ⁻ <i>araD139 Δ(argF-lac)U169 rspL150(Str^r) relA1flbB5301 deoC1 ptsF25 rbsR</i>	Casadaban (1976)
MHR204	MC4100 <i>csgA2::Tn105</i>	Hammar <i>et al.</i> (1996)
MHR263	MC4100 <i>recA csgB2</i>	Hammar <i>et al.</i> (1996)
MHR222	MC4100 <i>csgB1::Tn104</i>	Hammar <i>et al.</i> (1996)
Plasmids		
pMAL-p2	MBP fusion vector	New England Biolabs
pKK223-3	expression vector	Pharmacia Biotech
pZB-MB2	portion of <i>csgB</i> amplified with primers BNII-73 and BCII-74, fused to <i>malE</i> in <i>Asp700</i> and <i>HindIII</i> sites of pMAL-p2	this work
pZB-MB3	portion of <i>csgB</i> amplified with primers BNII-73 and BCIII-401, fused to <i>malE</i> in <i>Asp700</i> and <i>HindIII</i> sites of pMAL-p2	this work
pZB-MB4	portion of <i>csgB</i> amplified with primers BNII-73 and BCIV-402, fused to <i>malE</i> in <i>Asp700</i> and <i>HindIII</i> sites of pMAL-p2	this work
pZB-MB8	portion of <i>csgB</i> amplified with primers BNVIII-4601 and BCII-74, fused to <i>malE</i> in <i>Asp700</i> and <i>HindIII</i> sites of pMAL-p2	this work
pZB-MB9	portion of <i>csgB</i> amplified with primers BNIX-4602 and BCII-74, fused to <i>malE</i> in <i>Asp700</i> and <i>HindIII</i> sites of pMAL-p2	this work
pZB-KB1	entire <i>csgB</i> amplified with primers BNIV-802 and BCII-74 cloned in <i>EcoRI</i> and <i>HindIII</i> sites of pKK223-3	this work
Antibodies		
ZB-αA	purified anti-CsgA polyclonal Ab	Hammar <i>et al.</i> (1996)
ZB-αB	purified anti-CsgB peptide Ab	this work
αMalE	anti-MBP monoclonal antibody	New England Biolabs

were seen. When pZB-KB1 was introduced into MHR222, a strain unable to express CsgA as well as CsgB due to a polar insertion in *csgB*, colonies of MHR222/pZB-KB1 were stained with Congo red on a YESCA agar plate under curli-proficient condition (data not shown). Large quantities of cottony growth on the surface of the bacterial cell were seen under the electron microscope. No distinct structure was detected at lower magnification (Figure 3B). However, at high magnification, short polymers were clearly seen (Figure 3C) that reacted with the anti-CsgB antibody and therefore were decorated with gold particles (Figure 3D). These outgrowths, however, lacked the filamentous structure typical of curli from the wild-type strain MC4100. Overexpression of native CsgB in strain MHR222 also resulted in the formation of SDS-insoluble polymers which remained in the slot reacting with the CsgB-specific antibody (Figure 2, lane 5-), and were possible to depolymerize by 90% formic acid treatment (Figure 2, lane 6+). Thus the cell surface structures resulting from polymerized CsgB, when this protein is overexpressed, are insoluble, like wild-type curli, and able to bind Congo red.

An MBP-CsgB fusion protein triggers in vivo assembly of Bfco organelles that are morphologically different from wild-type curli

In order to prevent CsgB secretion and assembly into curli, we attempted to anchor CsgB in the outer membrane by fusing it to the periplasmic MBP. To this end, we generated a 339 bp PCR fragment corresponding to codons 21–131 of the *csgB* gene of MC4100, and fused it to the *malE* gene of pMAL-p2, resulting in pZB-MB2 (Table I, Figure 4). This plasmid codes for a fusion protein MBP–

Fig. 2. Western immunoblots of native CsgB and overexpressed CsgB in *E. coli*. MC4100, MHR263/pZB-KB1, MHR222/pZB-KB1 and MHR263 were grown on YESCA plates for >48 h at 28°C. Cells were loaded with (+) or without (–) prior formic acid treatment, and proteins were separated by 15% SDS–PAGE, transferred onto blotting membrane and analysed with CsgB-specific antibody ZB-αB. The locations of the slot and CsgB monomer are indicated on the left. Molecular markers are on the right in kDa.

CsgBII where the CsgB portion lacks the 20 amino-terminal amino acids of the mature protein. The *E. coli* strain MHR263, lacking expression of native CsgB, was transformed with pZB-MB2 and pMAL-p2 respectively, and grown on YESCA plates containing 100 µg/ml carbenicillin and 0.1 mM IPTG at 28°C. The curli-positive wild-type strain MC4100 was grown under the same

Fig. 3. Electron micrographs of cell surface-exposed CsgB in the *csgA* mutant strain MHR204 (A) and short CsgB polymers in MHR222/pZB-KB1 (B, C and D). Bacteria were grown on YESCA plates with or without carbenicillin under curli-proficient conditions for >48 h. Cells were either only negatively stained with 0.2% uranyl acetate (B and C) or combined with immunolabelling (A and D). Cells of MC4100 and MHR222/pZB-KB1 (A and D) were incubated with CsgB-specific antibody ZB- α B. Bacterial surface structures were labelled with protein A–gold particles (10 nm in diameter). Magnification: (A) $\times 100\ 000$; (B) $\times 30\ 000$; (C) $\times 200\ 000$; (D) $\times 60\ 000$.

Fig. 4. The *csg* gene cluster and derived CsgB protein derivatives. Arrows indicate the position and direction of the two *csg* promoters. Genes encoding proteins assumed to be exported are marked by boxes with closed promoter-proximal ends. The leftmost column shows the CsgB derivatives. The middle column shows their relative sizes; the repeated stars above the full-length CsgB show the positions of the 3 $\times$ 22 and 1 $\times$ 19 amino acid repeat region; the repeated short vertical bars above the MBP–CsgBIX indicate the location of the peptides, CsgB-PEP2 and CsgB-PEP1, against which the anti-CsgB peptide antiserum was raised. SP indicates the signal peptide. The rightmost column indicates the results of the complementation experiment in the *csgB* mutant MHR263.

Fig. 5. Electron micrographs of Bfco organelles in MHR263/pZB-MB2. MHR263/pZB-MB2 (A, B, C and E) and MHR263/pMAL-p2 (D) were grown on YESCA plates containing carbenicillin under curli-proficient conditions for >48 h. Cells were either only negatively stained with 0.2% uranyl acetate (A and D) or combined with immunolabelling (B, C and E). Cells of MHR263/pZB-MB2 were incubated with anti-CsgA (B), anti-CsgB (C) and anti-MBP (E) antibodies respectively. Bacterial surface structures were labelled with protein A–gold particles (10 nm in diameter). Magnification: (A), (B), (C) and (E) $\times 60\ 000$; (D) $\times 10\ 000$.

conditions without antibiotic and inducer. After 36 h of incubation, colonies of MC4100 and MHR263/pZB-MB2 turned red due to binding of the dye Congo red present in the YESCA plates. However, MHR263/pZB-MB2 colonies were stained less red than those of MC4100 (data not shown). Since curli expression is associated with a Congo red-binding phenotype, we speculated that strain MHR263/pZB-MB2 might be able to produce curli-related surface structures. To test this, bacteria harvested after >48 h were examined by electron microscopy (Figure 5). Organelles were present in MHR263/pZB-MB2 (Figure 5A) but not in MHR263/pMAL-p2 (Figure 5D). Strikingly, these organelles were morphologically very different from the curli fibres expressed by wild-type strain MC4100 (Figure 1). At high magnification, the organelles on MHR263/pZB-MB2 appeared as distinctly curved and loosely aggregated, 10–15 nm thick (the gold particles provided a standard of 10 nm in diameter) highly ordered fibres (Figure 5B, C and E), in contrast to the irregular branching masses of much less distinct, and much thinner (2 nm) wild-type curli fibres (Figure 1). The morphologic-

Fig. 6. Western blot of MBP-CsgBII expressed in the non-polar *csgB* mutant MHR263 and the polar *csgA*, *csgB* double mutant MHR222. MHR222/pZB-MB2 (lane 1), MHR263/pZB-MB2 (lane 2) and MHR263/pMAL-p2 (lane 3) were grown on YESCA plates containing carbenicillin for >48 h at 28°C. Cells were lysed by boiling in protein sample buffer, and proteins were separated with 15% SDS-PAGE before being transferred onto blotting membrane and analysed with monoclonal and anti-MBP antibody. Locations of the slot, MBP-CsgBII and MBP are indicated on the left. Molecular markers are shown on the right in kDa.

ally novel surface organelles on MHR263/pZB-MB2 were decorated symmetrically with gold particles along their length (Figure 5B) when the intact cells were incubated with the affinity-purified CsgA-specific antibody ZB- α A (Hammar *et al.*, 1996), but not with CsgB-specific antibody ZB- α B (Figure 5C) or an anti-MBP monoclonal antibody (Figure 5E).

In a Western blot, *in vivo* expressed MBP-CsgBII interacted with anti-MBP serum (Figure 6). When expressed in the *csgA*, *csgB* mutant strain MHR222, MBP-CsgBII was totally soluble in SDS (Figure 6, lane 1). However, when the same fusion protein was expressed in the CsgA-expressing strain, MHR263, under the same conditions, some MBP-CsgBII remained in the slot (Figure 6, lane 2). MHR263/pMAL-p2 was analysed as a control shown in Figure 6, lane 3. These data suggest that MBP-CsgBII by itself is unable to form SDS-resistant polymers but can stably interact with polymerized CsgA. Also, the fusion protein expressed in the absence of CsgA appeared to be more susceptible to *in vivo* proteolytic degradation.

Since the organelles observed were produced by a strain lacking a functional *csgB* gene, and they were labelled with anti-CsgA but not anti-CsgB antibodies, we interpret these novel organelles as being built up of CsgA subunits, free from CsgB protein, namely Bfco organelles.

MBP-CsgBII localizes to one of the two bacterial poles as do Bfco organelles

Unlike wild-type curli fibres, Bfco organelles resulting from the presence of CsgB fused to MBP appeared at only one of the two bacterial poles (Figure 7). Since it has been demonstrated that MBP is located predominantly in the periplasmic space at the bacterial pole (Dietzel *et al.*, 1978; Boos and Staehelin, 1981; Maddock and Shapiro, 1993), we wondered if polar assembly of Bfco organelles was due to polar location of MBP-CsgBII. As shown in Figure 5C, the poles containing Bfco organelles were also specifically surface labelled with gold particles when the ZB- α B antibody was probed. In contrast, no surface labelling was seen when the anti-MBP monoclonal antibody was used (Figure 5E), suggesting that the CsgB

Fig. 7. Electron micrographs of the polar distribution of Bfco organelles in MHR263/pZB-MB2. Cells were grown on YESCA plates with carbenicillin under curli-proficient conditions for >48 h. They were incubated with CsgA-specific antibody ZB- α A. Structures on the cell surface were labelled with protein A-gold particles (10 nm in diameter) and negatively stained. Magnification: (A), (D) and (E) $\times 60\,000$; (B) $\times 120\,000$; (C) $\times 100\,000$.

portion in the fusion is exposed on the cell surface, whereas its fusion partner MBP is not. Since MBP is a known periplasmic protein, it may be that this portion of the fusion protein is retained in the periplasm because of its bulky form. Thus, MBP-CsgBII goes to one of the two bacterial poles where the CsgB portion of the fusion protein can traverse the outer membrane and act as a membrane-bound nucleator for CsgA polymerization into Bfco fibres.

Fibronectin-binding properties of fibres containing CsgA, CsgB or both proteins

Curliated *E. coli* bind fibronectin while *E. coli* knockout mutants in either *csgA* or *csgB* do not. Depolymerized curli subunit proteins also bind fibronectin in a filter binding assay (Olsen *et al.*, 1993). Since CsgB is predicted to have a molecular weight similar to CsgA, and in view of the present finding that CsgB is a constituent of wild-type curli, it is possible that CsgB alone or in addition to CsgA contributes to fibronectin binding. A non-radiolabel assay of fibronectin binding to *Staphylococcus aureus* has been shown to yield a satisfactory signal-to-background ratio, which was comparable with a radiolabel assay (Flock *et al.*, 1996). In this study, we used the same method with some modifications, described in Materials and methods. As shown in Figure 8, *E. coli* MHR263/pZB-KB1 producing curli with a higher content of CsgB bound fibro-

Fig. 8. Binding of *E. coli* to plastic coated with fibronectin. Bacterial cells were grown on YESCA plates under curli-proficient conditions for >48 h. Microtitre wells were coated with 100 µl of bovine fibronectin at concentrations ranging from 0.08 to 10 µg/ml. Two hundred µl of 4×10^8 cells/ml bacteria ($OD_{600} = 0.94-0.95$) were added. Adherent bacteria were determined with a microplate reader (A_{405}). Each value is the mean and standard error of one (MHR204, MHR263/pMAL-p2) or two (MC4100, MHR263/pZB-KB1, MHR222/pZB-KB1, MHR263/pZB-MB2) independent triplicate assays. (A) The relative adherence of bacteria from each strain binding to fibronectin at different concentrations. (B) Two hundred µl of 4×10^8 cells/ml of bacteria from each strain binding to fibronectin at the concentration of 5 µg/ml.

nectin at a level higher than MC4100, the wild-type curli-producing strain. MHR222/pZB-KB1 lacking CsgA and hyperproducing CsgB in the form of short polymers also bound fibronectin, albeit at a lower level. *Escherichia coli* expressing Bfco organelles containing only CsgA bound significantly less fibronectin than MC4100. Strains deficient in either *csgA* or *csgB*, such as MHR204 and MHR263/pMAL-p2, that express no surface organelles, did not bind fibronectin at all. Thus, the presence of CsgB in polymerized fibres appears to contribute to fibronectin binding.

The C-terminal 110 amino acid pseudo repeat region is essential for triggering polymerization of CsgA in vivo

To localize further the region of CsgB capable of triggering the polymerization of CsgA, various portions of *csgB* were amplified and cloned into pMAL-p2, yielding a series of *malE::csgB* fusion derivatives (Table I, Figure 4). MBP and the fusion proteins (MBP-BII, MBP-BIII, MBP-BIV, MBP-BVIII and MBP-BIX) were expressed in the host *E. coli* strain XL1-Blue after induction with 0.3 mM IPTG. Thereafter, the respective fusion proteins were affinity purified on an amylose resin column. The purified products of these *malE::csgB* fusion clones were run on a 15% SDS-polyacrylamide gel as shown in Figure 9. MBP has an M_r of 42 kDa (Figure 9, lane 1), whereas the individual fusion proteins were all larger, but protein degradation occurred for some of them, resulting in multiple protein bands. The *malE::csgB* fusion plasmids were transformed into the *csgB* mutant strain MHR263, and the resulting transformants incubated on YESCA plates with carbenicillin at 28°C for >48 h. Except for MHR263/pZB-MB2, no other strains gave rise to Congo red-binding colonies and no surface structures were visible under the electron microscope (data not shown). Western immunoblotting assays verified that the fusions reacted with both anti-MBP monoclonal antibody and anti-CsgB antiserum (Figure 10). However, protein degradation occurred in all cases. Assuming that the largest immuno-

Fig. 9. SDS-PAGE chromatography of affinity-purified MBP and MBP-CsgB fusion proteins expressed in *E. coli* XL1-Blue. Bacterial cells harbouring pMAL-p2, pZB-MB2, pZB-MB3, pZB-MB4, pZB-MB8 and pZB-MB9 were grown in LB medium with 50 µg/mg carbenicillin. The fusion proteins were induced with 0.3 mM IPTG and affinity-purified as described in Materials and methods. Proteins were subjected to 15% SDS-PAGE and stained with Coomassie brilliant blue. MBP (lane 1) is the standard with a mol. wt of 42 kDa. Lanes 2-6 were MBP-CsgBII, MBP-CsgBIII, MBP-CsgBIV, MBP-CsgBVIII and MBP-CsgBIX.

Fig. 10. Western blot of the MBP and MBP-CsgB fusion proteins expressed in MHR263. Bacterial cells harbouring pMAL-p2, pZB-MB2, pZB-MB3, pZB-MB4, pZB-MB8 and pZB-MB9 were grown on YESCA plates with 100 µg/mg carbenicillin and 0.1 mM IPTG for >48 h at 28°C. Cells were boiled in sample buffer. Proteins were separated by 15% SDS-PAGE, transferred to nitrocellulose membrane and analysed with anti-MBP and anti-CsgB antibodies. The molecular weight of MBP is ~42 kDa and is indicated on the left. Molecular markers are shown on the right in kDa.

reactive protein band for MBP-CsgBII (Figure 10, lane 2) corresponds to the full-length nucleator active version of the fusion, the largest bands for MBP-CsgBIII and MBP-CsgBVIII (Figure 10, lanes 3 and 5) run with a molecular weight corresponding to the complete fusion proteins. Low amounts of the apparent full-length protein were also observed for *in vivo* expressed MBP-CsgBIV

and MBP-CsgBIX (Figure 10, lanes 4 and 6). Therefore, all the fusion proteins were expressed in MHR263. Since MBP-CsgBVIII lacks 23 N-terminal CsgB residues relative to MBP-CsgBII, and MBP-CsgBIII lacks 43 C-terminal CsgB residues, we conclude that most of the 110 CsgB residues in MBP-CsgBII are needed for triggering the polymerization of CsgA. This region of CsgB in MBP-CsgBII contains the complete set of pseudo repeats also seen in CsgA (Hammar *et al.*, 1996).

Discussion

We recently have demonstrated that the assembly of curli fibres in *E. coli* occurs outside the microbe through precipitation of secreted soluble CsgA subunit protein into thin aggregated fibres (Hammar *et al.*, 1996). This reaction requires the CsgB protein that shows extensive sequence homology to CsgA. It was suggested that CsgB acted as a nucleator for the polymerization of soluble CsgA monomer, since these fibres were formed intercellularly on cells expressing only CsgB, provided such cells were grown in close proximity to cells lacking CsgB, and therefore secreting soluble CsgA. To explain this inter-bacterial fibre formation, we speculated that CsgB might be surface exposed on the bacteria, acting as a nucleator or an anchor.

In this study, by using immunoelectron microscopy and immunoblotting assays with the affinity-purified specific anti-CsgB and anti-CsgA antibodies, ZB- α B and ZB- α A, we demonstrate the capacity of CsgB to act as a nucleator, triggering the polymerization of CsgA monomers, forming wild-type curli fibres and Bfco organelles. We show that CsgB expressed at wild-type levels in the absence of CsgA is located on the bacterial cell surface and, when expressed together with CsgA, it also appears along the length of the curli fibres. When CsgB was overexpressed, more CsgB molecules were present in the curli-like fibres without changing the morphology compared with wild-type curli. Interestingly, in the absence of CsgA, overexpression of CsgB resulted in the formation of SDS-insoluble short CsgB polymers on the bacterial cell surface.

We suggest that, at wild-type levels, CsgB is translocated across the outer membrane, after which it can either bind to components on the exterior surface of the outer membrane or be engaged in polymerization with CsgA. We assume that the translocation of CsgB and CsgA across the outer membrane requires a specific protein translocator, the nature of which is unknown. It is possible that the concomitant translocation of CsgB and CsgA through the same translocator may allow CsgB-mediated nucleation during the secretion process, allowing CsgB to be pushed out from the cell surface by polymerized CsgA. CsgB in the polymerized fibre was often found at sites where curli fibres appeared to branch. Such branching would be possible to explain if CsgB contains multiple interaction sites with CsgA or if oligomeric forms of CsgB allow for multinucleation. Hence, when CsgB proteins are present in wild-type curli, the free site of an individual CsgB molecule or CsgB oligomer might act as a new nucleation site, triggering the polymerization of soluble CsgA, resulting in the formation of highly branched fibres.

Nucleation events may also occur with surface-bound CsgB for bacterial anchorage of the curli fibres.

When CsgB is fused to MBP, we believe that the bulky MBP portion of the fusion protein cannot pass through the outer membrane translocator, leading to a trans-membrane protein where only the CsgB portion is surface exposed. Such a surface-anchored nucleator can only trigger polymerization of CsgA molecules secreted through separate translocators, only allowing the growth of CsgA fibres from the distal end without the possibility of branching.

The presence of MBP in MBP-CsgBII not only results in the formation of highly ordered Bfco organelles lacking incorporated CsgB, but also affects the position of fibres on the bacterial cell. Thus, the Bfco fibres only appeared at one of the two bacterial poles. We believe that this polar location is due to a polar secretion of MBP-CsgBII, as has been shown for MBP alone (Dietzel *et al.*, 1978; Boos and Staehelin, 1981; Maddock and Shapiro, 1993). Asymmetric distribution of a number of bacterial proteins has been described in recent years (Kocks *et al.*, 1993; Shapiro and Losick, 1997). This asymmetry does not seem to depend on random insertion followed by non-random proteolysis, but rather on an asymmetric targeting of proteins within the cell. In the present study, we argue that MBP but not CsgB is targeted for polar secretion across the cytoplasmic membrane, causing the positional difference between Bfco organelles and wild-type curli fibres.

We speculate that the binding of MBP-CsgBII to CsgA induces CsgA to undergo a structural transition to a conformation that mimics that of CsgB. The conformationally altered CsgA is now able to bind the next soluble CsgA monomer and induce a similar conformational change. A repetition of this process eventually leads to the formation of long insoluble CsgA-containing Bfco organelles. The above model bears similarity with the proposed process through which the scrapie protein PrP^{Sc}, by binding to PrP^C, induces a conformational change of PrP^C (Glenn *et al.*, 1996; Huang *et al.*, 1996).

Biological structures frequently contain assemblies or complexes of several protein subunits. The high sequence homology between CsgA and CsgB suggests that both proteins possess some similar structural and functional characteristics for the formation of curli fibres. The finding here that CsgB is another component of curli is contrary to the original observation that CsgA was the only subunit protein found in a purified curli preparation (Collinson *et al.*, 1991; Olsen *et al.*, 1993). It is possible that CsgB is lost during the harsh curli preparation. Alternatively, CsgB might normally be present in curli in such low amounts that its presence was not detected, and was simply hidden beneath the CsgA protein band after depolymerization in 90% formic acid. The present data clearly demonstrate that CsgB is integrated into curli as a minor component, and not merely associated with the exterior surface of the fibres. Minor proteins are known to be present also in various fimbriae. In P fimbriae, these minor proteins are located at the tip of the organelles, fulfilling functions as adhesins, adaptors or possibly initiators for polymerization (Lindberg *et al.*, 1987; Kuehn *et al.*, 1992; Jacob-Dubuisson *et al.*, 1993). In Type 1 fimbriae, the

FimH adhesin was found both at the tip and along the pilus fibres (Ponniah *et al.*, 1991; Jones *et al.*, 1995).

Even though CsgA and CsgB show 30% identity and 49% similarity, and are built up of similar repeat motifs, they clearly behave quite differently. In the absence of CsgB, CsgA is secreted into the medium (Hammar *et al.*, 1996) and, even when it is overexpressed, no autoaggregates can be observed on the bacterial surface or in the culture medium (data not shown). CsgB is also translocated through the outer membrane, but is not secreted into the medium in the absence of CsgA, even when overexpressed (data not shown). It is possible that in the presence of CsgA, CsgB is secreted, but incorporated immediately into fibres due to its nucleating activity. Immunoelectron microscopy shown here reveals that CsgB is surface exposed in the absence of CsgA. This also has been verified recently by Lofrer *et al.*, 1997, where CsgB was found to be completely sensitive to proteinase K treatment of intact cells. Overexpression of CsgB in the absence of CsgA resulted in masses of short polymers on the bacterial surface. The polymers were SDS resistant and therefore reacting with CsgB-specific antibody at the top of the stacking gel. In contrast, CsgA expressed in the absence of CsgB remained completely SDS soluble. Thus, CsgB and CsgA are both translocated. However, CsgB produced in the absence of CsgA is prone to self-assemble, leading to a precipitation on the bacterial surface, whereas CsgA depends on CsgB for precipitation into curli fibres. It is intriguing but not all surprising that CsgB can act both as a nucleator protein for CsgA and by its itself polymerize into polymers. Recently, γ -tubulin was demonstrated to be both a nucleator for microtubule formation and to self-assemble into novel tubular structures in mammalian cells (Shu and Joshi, 1995). γ -tubulin is a new member of the tubulin gene family with up to 35% sequence identity to the classical α - and β -tubulins (Joshi *et al.*, 1992).

If CsgB acts as a true nucleator, it must be able to interact with CsgA. The sequence homology between CsgB and CsgA makes it likely that CsgB physically interacts with CsgA. Our evidence for such a direct interaction is as follows: (i) when CsgB is produced by itself at wild-type levels it is surface located, but when expressed together with CsgA in wild-type bacteria it is present in curli fibres, suggesting a CsgB–CsgA interaction; (ii) the MBP–CsgBII fusion protein was stabilized when CsgA was produced, and appeared in an SDS-resistant complex at the top of the gel only in the presence of CsgA; (iii) the polar expression of the nucleator CsgB as part of the MBP–CsgBII fusion results in polar assembly of CsgA-containing Bfco organelles.

The MBP–CsgBII fusion contains the 110 C-terminal residues of CsgB. The 20 N-terminal residues of mature CsgB are therefore not required for the nucleation function. These 110 amino acids contain the entire repeat region of the native CsgB. Since fusion protein MBP–CsgBVIII, lacking only an additional 23 amino acids from the N-terminal region including the first repeat, is totally inactive as a nucleator, we suggest that a complete repeat region is necessary for nucleator function. Since CsgA contains a very similar repeat region, it is reasonable to hypothesize that an interaction between the repeat regions of CsgB and CsgA induces a conformational change in

the latter protein leading to the formation of surface organelles.

Hydrophobic interactions are regarded as being important in a variety of microbial adhesion phenomena in both the environment and the host (Rosenberg and Doyle, 1990). Congo red binding has been used as an indication of surface hydrophobicity for certain pathogens (Surgalla and Beesley, 1969). In some strains of *E.coli*, cell surface hydrophobicity has been related to the presence of fimbriae (Ljungh and Wadstrom, 1982; Wadstrom *et al.*, 1986). Congo red binding has been shown to be tightly associated with the expression of curli fibres in *E.coli* (Hammar *et al.*, 1995). We have shown here that the structural properties of both CsgA-containing Bfco organelles and the short CsgB polymers are associated independently with a Congo red-binding phenotype, indicating that surface structures resulting from CsgA, free from CsgB, and CsgB, free from CsgA, are able to bind this dye. We do not know whether other *csg* gene products besides CsgA and CsgB may contribute to the Congo red-binding phenotype.

Fibronectin binding is one of the unique features of curli-expressing *E.coli* and *Salmonella* (Olsen *et al.*, 1989; Collinson *et al.*, 1991). Besides fibronectin, curli-expressing bacteria bind a series of host proteins such as laminin, plasminogen and human contact phase proteins (Arnqvist *et al.*, 1992; Sjöbring *et al.*, 1994; Ben Nasr *et al.*, 1996). The present data clearly demonstrate that binding of curli-expressing *E.coli* to fibronectin is influenced by the presence of CsgB protein in the fibres, because overexpression of CsgB in the presence of CsgA results in a higher level of fibronectin binding as compared with bacteria expressing wild-type curli. Cells expressing Bfco organelles or short CsgB polymers also bind fibronectin, but at a much lower level, suggesting that bacterial surface structures made up of either CsgA or CsgB were able to bind fibronectin. However, since cells expressing wild-type curli show a much higher fibronectin-binding capacity than cells expressing Bfco organelles, this indicates that CsgB, as an integral part of curli, contributes to fibronectin binding. Previous studies have shown that depolymerized curli monomers can bind fibronectin (Olsen *et al.*, 1993). Since CsgA, prior to this study, was thought to be the sole component of curli, it was suggested that binding was to monomeric CsgA. As the CsgB protein has the same molecular weight as CsgA, is a minor component of wild-type curli and can be depolymerized into monomers by formic acid treatment, fibronectin binding might be to monomeric CsgB.

To understand fully how CsgB mediates its nucleation function on CsgA and how this protein contributes to the adhesive property of curli, *in vitro* assembly from purified components is needed. However, before such studies can be initiated, it is necessary to characterize other *csg* genes involved in the formation of curli and see if their gene products may affect either CsgA or CsgB post-translationally.

Materials and methods

Experimental materials

Restriction enzymes and DNA-modifying enzymes were used as recommended by the manufacturer (Boehringer Mannheim, New England

Table II. Oligonucleotides used as primers in this study

Name	Sequence	Position in <i>csg</i> sequence ^a
BNII-73	5'GGCGCGAGAAAGTCTTCATTTA	3349
BCII-74	5'TCGAATGAAGCTTAACGTTGTGT	3688
BCIII-401	5'GCATAAGCTTAAGCACCTTGCGA	3558
BCIV-402	5'TGCAAAAGCTTAATCTCCTGTCTG	3495
BNVIII-4601	5'GCGAAGAGGTTCAAACTTTTGG	3424
BNIX-4602	5'ATGGAAGTATTTTCGCAAGGTGC	3538
BNIV-802	5'CGGAATTCCAGGGTGACAACAT	3218
MalE*	5'GGTCGTCAGACTGTGATGAAGCC	–
tac/trp-605	5'TGAGCTGTTGACAATTAATCATCG	–

^aThe *csg* sequence is available from DDBJ/EMBL/GenBank under the accession No. x90754.

Biolabs). Amylose resin was purchased from New England Biolabs. Oligonucleotides, CNBr-activated Sepharose 4B and pre-stained protein marker were purchased from Pharmacia Biotech. Protein A-conjugated gold particles, Formvar-coated grids, carbenicillin, IPTG, 5-bromo-4-chloro-3-indolyl- β -D-galactoside (X-gal), nitroblue tetrazolium chloride/5-bromo-4-chloro-3-indolyl phosphate (NBT/BICP) and bovine fibronectin were purchased from Sigma Chemical Co.

Bacterial strains, plasmids and antibodies

The *E. coli* strains, plasmids and antibodies used in this study are described in Table I.

Construction of plasmids

Oligonucleotide primers used to amplify different portions of the *csgB* gene are listed in Table II. PCR was carried out in a 100 μ l reaction containing 50 pmol of each primer and 250 μ M of each dNTP in *Taq* reaction buffer [10 mM Tris-HCl (pH 8.3), 1.5 mM MgCl₂, 50 mM KCl]. Crude chromosomal DNA isolated from wild-type strain MC4100 was used as template. Amplification was performed on an automatic thermocycler (Perkin Elmer Cetus). Each reaction was electrophoresed through a 1.5% agarose gel to verify production of the appropriate fragments. The PCR products were excised and purified using GeneClean (BioRad). PCR products used for the constructs (Table I) were digested sequentially with restriction enzymes. Following heat inactivation and phenol extraction, the digested PCR products were purified with GeneClean again. Plasmids pMAL-p2 and pKK223-3 were digested sequentially and purified. Ligation reactions were carried out using standard protocols (Miller, 1972). Transformants were screened by plating duplicates on LB agar with 100 μ g/ml carbenicillin, 25 μ g/ml X-gal and 0.25 mM IPTG, when working with pMAL-p2. Candidate colonies were checked by PCR with appropriate primers. Plasmid DNA was isolated from the positive clones and determined by dideoxynucleotide chain termination (Sequenase). Oligonucleotides used for sequencing were primers of MalE* for *malE* fusion constructs (pZB-MB2, pZB-MB3, pZB-MB4, pZB-MB8 and pZB-MB9) and tac/trp-605 for pZB-KB1.

Purification of MBP and MBP-CsgB fusion proteins

MBP and fusion proteins were purified by affinity chromatography. An overnight broth culture of *E. coli* strain XL1-Blue, harbouring pMAL-p2, pZB-MB2, pZB-MB3, pZB-MB4, pZB-MB8 and pZB-MB9 respectively, was inoculated into 100 volumes of LB broth plus 50 μ g/ml carbenicillin, and incubated at 37°C until the OD₆₀₀ reached 0.6. The expression of proteins was induced by 0.3 mM IPTG, and bacterial cells were incubated for an additional 2 h. Cells were pelleted by centrifugation and the periplasmic contents containing the fusion protein were released by osmotic shock treatment (Neu and Heppel, 1965). One volume of 5 $\times$ column buffer [100 mM Tris-HCl (pH 7.4), 1 M NaCl, 5 mM EDTA, 50 mM 2-mercaptoethanol, 5 mM NaN₃] was added to four volumes of the supernatant (osmotic shock fluid). The buffered osmotic shock fluid was applied to a column (Econo-Column, 1.5 $\times$ 5 cm, Bio-Rad) packed with amylose resin at 0.5 ml/min. After intensive washing with column buffer, MBP and MBP-CsgB fusion proteins were eluted with 10 mM maltose and collected into fractions. An aliquot from each fraction was examined by electrophoresis through a 15% SDS-polyacrylamide gel. Proteins were visualized by Coomassie blue staining.

Purification of polyclonal antibodies

ZB- α A. About 10 ml of CNBr-activated Sepharose 4B gel were prepared according to the supplier (Pharmacia Biotech, Sweden). Twenty mg of MBP were coupled onto the beads by incubating overnight at 4°C in coupling buffer [0.1 M NaHCO₃ (pH 8.3), 0.5 M NaCl]. The adsorbent was packed into a low-pressure column (C-column, Pharmacia Biotech). Ten ml of rabbit antiserum raised against MBP-CsgAIII (Hammar *et al.*, 1996) was passed down the column at 6 ml/h. All the sera which could not be adsorbed were collected into fractions. Antisera in the fractions were again adsorbed extensively against the lysates from *E. coli* strain MC4100 grown at 37°C, and therefore not expressing *csg* genes products, resulting in the specific anti-CsgA polyclonal antibody, ZB- α A (ZB-AIII, Hammar *et al.*, 1996). ZB- α A was used for immunoelectron microscopy and immunoblot at a dilution of 1:200–1:2000.

ZB- α B. Two peptides were designed according to the deduced amino acid sequence of the *csgB* gene (CsgB-PEP2, aa 54–69 and CsgB-PEP-1, aa 111–131). The peptides were synthesized commercially and used to immunize rabbits (TANA Lab, Texas). Ten ml of CNBr-activated Sepharose 4B gel were prepared according to the supplier (Pharmacia Biotech, Sweden). Two mg of the mixture of the two peptides were coupled to the beads by incubation overnight at 4°C in coupling buffer [0.1 M NaHCO₃ (pH 8.3), 0.5 M NaCl]. The adsorbent was packed into a low-pressure column (C-column, Pharmacia Biotech). Five ml of rabbit antiserum were passed down the column at 0.25 ml/min. After intensive washing with phosphate-buffered saline (PBS), the specific anti-CsgB immunoglobulins, ZB- α B, which bound to the beads were eluted with glycine, HCl, NaCl buffer, and collected into fractions which were neutralized with Tris base buffer. ZB- α B was used for immunoelectron microscopy and immunoblotting at a dilution of 1:50–1:500.

Immunoblot

Bacterial cells were incubated on YESCA plates (Hammar *et al.*, 1995) for >48 h at 28°C. A suspension of bacterial cells in protein sample buffer was boiled for 10 min, and the constituent proteins were separated on a 15% SDS-polyacrylamide slab gel (Swank and Munkres, 1971). Immunoblot experiments were performed essentially as described earlier (Towbin *et al.*, 1979). The proteins were then transferred onto a nitrocellulose membrane (0.45 μ m pore size in roller form, MSI, USA) at 100 V for 60 min. The electrophoretic blot was soaked in 5% non-fat milk in TBS [10 mM Tris-HCl (pH 7.5), 150 mM NaCl] for at least 30 min. The membrane was incubated with purified ZB- α A at 1:1000–1:2000 dilution, ZB- α B at 1:300–1:500 dilution and anti-MBP monoclonal antibody at 1:10 000 dilution in TBS containing 0.5% bovine serum albumin (BSA) for 60 min at 37°C. Alkaline phosphatase-conjugated goat anti-rabbit IgG (Dakopatts AB) at 1:4000 dilution was used as the second antibody. Coloured alkaline phosphatase products on the nitrocellulose sheet were developed with NBT/BICP in alkaline phosphatase buffer [0.1 M Tris-HCl (pH 9.5), 0.1 M NaCl, 5 mM MgCl₂] at room temperature.

Electron microscopy

Electron microscopy was performed using Phillips CM201, JEOL-1200 and Zeiss EM109 electron microscopes at 60–80 kV with copper or nickel grids (200 and 300 mesh) coated with thin films of 2% Formvar. Bacterial colonies after 48 h incubation were overlaid with PBS (pH 7.4), and the cell suspension was allowed to sediment for 2 min on a grid. After washing with PBS followed by distilled water, the specimen was negatively stained with 0.2% uranyl acetate and air dried before performing the electron-microscopic study. For immunoelectron microscopy, specimens were blocked with 1% BSA/PBS, and then incubated with ZB- α A (1:200 dilution in 1% BSA/PBS), ZB- α B (1:50 dilution) or anti-MBP antiserum (1:1000) for 60 min at 37°C. Following washing with PBS, the specimen was incubated with gold particle-labelled protein A (10 nm colloidal gold particles) for 30 min at 37°C, rinsed with PBS followed by distilled water and negatively stained.

Assay of fibronectin binding

Quantitation of non-radiolabelled fibronectin binding to *E. coli* has been modified according to the method described earlier (Flock *et al.*, 1996). Bacteria were grown on YESCA agar plates under curli-proficient conditions for 48 h. Microtitre wells (Dynatech laboratories, Inc., Chantilly, VA) were coated with 100 μ l of bovine fibronectin (Sigma Chemical Co., St. Louis, MO), ranging from 0.08 to 10 μ g/ml, at 4°C overnight and at room temperature for an additional 2 h. After blocking with 2% BSA in PBST (PBS with 0.05% Tween-20) for 2 h at room temperature, 200 μ l of 4 $\times$ 10⁸ cells/ml (OD₆₀₀ = 0.94–0.95) in PBST

were constantly added to the wells and incubated at room temperature for 3 h. The wells were washed, and the amount of light absorbance of the adherent bacteria was determined with a microplate reader (A_{405}).

Acknowledgements

We thank Mårten Hammar for providing *E.coli* MHR# strains. We are grateful to Mikael Rhen for critical reading of the manuscript. We appreciate the help of Dr J.-I.Flock and Ms I.Löfving-Arvholm (Huddinge Hospital) in setting up the fibronectin-binding assay. Professor T.Hökfelt (Karolinska Institute) is acknowledged for allowing use of their JOEL-1200 electron microscope for some of the EM work. Dr J.A.Ampofo (Institute of Aquatic Biology, Ghana) is acknowledged for reading the manuscript. Z.B. was supported by a PhD fellowship from the Karolinska Institute. This work was supported by grants from the Swedish Medical Research Council (16x-10843), the Swedish Natural Science Research Council (3373-309), the Göran Gustafssons Foundation of Natural and Medical Science and an unrestricted grant from Bristol-Myers Squibbs to S.N.

References

- Arnqvist, A., Olsen, A., Pfeifer, J., Russell, D.G. and Normark, S. (1992) The Crl protein activates cryptic genes for curli formation and fibronectin binding in *Escherichia coli* HB101. *Mol. Microbiol.*, **6**, 2443–2452.
- Arnqvist, A., Olsen, A. and Normark, S. (1994) Sigma^S-dependent growth-phase induction of the *csgBA* promoter in *Escherichia coli* can be achieved *in vivo* by sigma⁷⁰ in the absence of the nucleoid-associated protein H-NS. *Mol. Microbiol.*, **13**, 1021–1032.
- Ben Nasr, A., Olsen, A., Sjöbring, U., Mulleresterl, W. and Björck, L. (1996) Assembly of human contact phase proteins and release of bradykinin at the surface of curli-expressing *Escherichia coli*. *Mol. Microbiol.*, **20**, 927–935.
- Boos, W. and Staehelin, A.L. (1981) Ultrastructural localization of the maltose-binding protein within the cell envelope of *Escherichia coli*. *Arch. Microbiol.*, **129**, 240–246.
- Brinton, C.C., Jr (1965) The structure, function, synthesis and genetic control of bacterial pili and a molecular model for DNA and RNA transport in Gram negative bacteria. *Trans. NY Acad. Sci.*, **27**, 1003–1054.
- Bullock, W.O., Fernandez, J.M. and Short, J.M. (1987) X11-Blue: a high efficiency plasmid transforming *recA* *Escherichia coli* strain with beta-galactosidase selection. *BioTechniques*, **5**, 376–378.
- Casadaban, M.J. (1976) Transposition and fusion of the *lac* genes to selected promoters in *Escherichia coli* using bacteriophage lambda and Mu. *J. Mol. Biol.*, **104**, 541–555.
- Collinson, S.K., Emödy, L., Muller, K.H., Trust, T.J. and Kay, W.W. (1991) Purification and characterization of thin, aggregative fimbriae from *Salmonella enteritidis*. *J. Bacteriol.*, **173**, 4773–4781.
- Dietzel, I., Kolb, V. and Boos, W. (1978) Pole cap formation in *Escherichia coli* following induction of the maltose-binding protein. *Arch. Microbiol.*, **118**, 207–218.
- Duguid, J.P., Smith, I.W., Dempster, G. and Edmunds, P.N. (1955) Non-flagella filamentous appendages 'fimbriae' and haemagglutinating activity in *Bacterium coli*. *J. Pathol. Bacteriol.*, **70**, 335–348.
- Flock, J.I., Hienz, S.A., Heimdahl, A. and Schennings, T. (1996) Reconsideration of the role of fibronectin binding in endocarditis caused by *Staphylococcus aureus*. *Infect. Immun.*, **64**, 1876–1878.
- Glenn, C. et al. (1996) Evidence for the conformation of the pathologic isoform of the prion protein enciphering and propagating prion. *Science*, **274**, 2079–2082.
- Hammar, M., Arnqvist, A., Bian, Z., Olsen, A. and Normark, S. (1995) Expression of two *csg* operons is required for production of fibronectin and congo red-binding curli polymers in *Escherichia coli* K-12. *Mol. Microbiol.*, **18**, 661–670.
- Hammar, M., Bian, Z. and Normark, S. (1996) Nucleator-dependent intercellular assembly of adhesive curli organelles in *Escherichia coli*. *Proc. Natl Acad. Sci. USA*, **93**, 6562–6566.
- Huang, Z., Prusiner, S.B. and Cohen, F.E. (1996) Structures of prion proteins and conformational models for prion diseases. *Curr. Top. Microbiol. Immunol.*, **207**, 49–67.
- Hultgren, S.J., Normark, S. and Abraham, S.N. (1991) Chaperone-assisted assembly and molecular architecture of adhesive pili. *Annu. Rev. Microbiol.*, **45**, 383–415.
- Jacob-Dubuisson, F., Heuser, J., Dodson, K., Normark, S. and Hultgren, S. (1993) Initiation of assembly and association of the structural elements of a bacterial pilus depend on two specialized tip proteins. *EMBO J.*, **12**, 837–847.
- Jones, C.H., Pinkner, J.S., Nicholes, A.V., Slonim, L.N., Abraham, S.N. and Hultgren, S.J. (1993) FimC is a periplasmic PapD-like chaperone that directs assembly of type 1 pili in bacteria. *Proc. Natl Acad. Sci. USA*, **90**, 8397–8401.
- Jones, C.H., Pinkner, J.S., Roth, R., Heuser, J., Nicholes, A.V., Abraham, S.N. and Hultgren, S.J. (1995) FimH adhesin of type 1 pili is assembled into a fibrillar tip structure in the *Enterobacteriaceae*. *Proc. Natl Acad. Sci. USA*, **92**, 2081–2085.
- Joshi, H.C., Palacios, M.J., McNamara, L. and Cleveland, D.W. (1992) Gamma-tubulin is a centrosomal protein required for cell cycle-dependent microtubule nucleation. *Nature*, **356**, 80–83.
- Kocks, C., Hellio, R., Gounon, P., Ohayon, H. and Cossart, P. (1993) Polarized distribution of *Listeria monocytogenes* surface protein ActA at the site of directional actin assembly. *J. Cell Sci.*, **105**, 699–710.
- Kuehn, M.J., Heuser, J., Normark, S. and Hultgren, S.J. (1992) P pili in uropathogenic *E.coli* are composite fibres with distinct fibrillar adhesive tips. *Nature*, **356**, 252–255.
- Lindberg, F., Lund, B., Johansson, L. and Normark, S. (1987) Localization of the receptor-binding protein adhesin at the tip of the bacterial pilus. *Nature*, **328**, 84–87.
- Ljungh, A. and Wadstrom, T. (1982) Salt aggregation test for measuring cell surface hydrophobicity of urinary *Escherichia coli*. *Eur. J. Clin. Microbiol.*, **1**, 388–393.
- Loferer, H., Hammar, M. and Normark, S. (1997) Availability of the fiber subunit CsgA and the nucleator protein CsgB during assembly of fibronectin-binding curli is limited by the intracellular concentration of the novel lipoprotein CsgG. *Mol. Microbiol.*, in press.
- Maddock, J.R. and Shapiro, L. (1993) Polar location of the chemoreceptor complex in the *Escherichia coli* cell. *Science*, **259**, 1717–1723.
- Miller, J.H. (1972) *Experiments in Molecular Genetics*. Cold Spring Harbor Laboratory Press, Cold Spring Harbor, NY.
- Neu, H.C. and Heppel, L.A. (1965) The release of enzymes from *Escherichia coli* by osmotic shock and during the formation of spheroplasts. *J. Biol. Chem.*, **240**, 3685–3692.
- Olsen, A., Jonsson, A. and Normark, S. (1989) Fibronectin binding mediated by a novel class of surface organelles on *Escherichia coli*. *Nature*, **338**, 652–655.
- Olsen, A., Arnqvist, A., Hammar, M., Sukupolvi, S. and Normark, S. (1993) The RpoS sigma factor relieves H-NS-mediated transcriptional repression of *csgA*, the subunit gene of fibronectin-binding curli in *Escherichia coli*. *Mol. Microbiol.*, **7**, 523–536.
- Ponniah, S., Endres, R.O., Hasty, D.L. and Abraham, S.N. (1991) Fragmentation of *Escherichia coli* type 1 fimbriae exposes cryptic D-mannose-binding sites. *J. Bacteriol.*, **173**, 4195–4202.
- Rosenberg, M. and Doyle, R.J. (1990) Microbial cell surface hydrophobicity: history, measurement and significance. In Rosenberg, M. and Doyle, R.J. (eds), *Microbial Cell Surface Hydrophobicity*. ASM Publications, Washington, DC, pp. 1–37.
- Shapiro, L. and Losick, R. (1997) Protein localization and cell fate in bacteria. *Science*, **276**, 712–718.
- Shu, H.B. and Joshi, H.C. (1995) Gamma-tubulin can both nucleate microtubule assembly and self-assemble into novel tubular structures in mammalian cells. *J. Cell Biol.*, **130**, 1137–1147.
- Sjöbring, U., Pohl, G. and Olsen, A. (1994) Plasminogen, absorbed by *Escherichia coli* expressing curli or by *Salmonella enteritidis* expressing thin aggregative fimbriae, can be activated by simultaneously captured tissue-type plasminogen activator (t-PA). *Mol. Microbiol.*, **14**, 443–452.
- Sukupolvi, S., Edelstein, A., Rhen, M., Normark, S. and Pfeifer, J. (1997) Development of a murine model of chronic *Salmonella* infection. *Infect. Immun.*, **65**, 838–842.
- Surgalla, M.J. and Beesley, E.D. (1969) Congo red-agar plating medium for detecting pigmentation in *Pasteurella pestis*. *Appl. Microbiol.*, **18**, 834–837.
- Swank, R.T. and Munkres, K.D. (1971) Molecular weight analysis of oligopeptides by electrophoresis in polyacrylamide gel with sodium dodecyl sulfate. *Anal. Biochem.*, **39**, 462–477.
- Towbin, H., Staehelin, T. and Gordon, J. (1979) Electrophoretic transfer of proteins from polyacrylamide gels to nitrocellulose sheets: procedure and some applications. *Proc. Natl Acad. Sci. USA*, **76**, 4350–4354.
- Wadstrom, T., Adegbola, R.A., Baloda, S.B., Ljungh, A., Sethi, S.K. and Yuk, Y.R. (1986) Non-haemagglutinating fimbriae of enteropathogenic *Escherichia coli* (EPEC). *Zentralbl. Bakteriol. Mikrobiol. Hyg., Ser. A*, **261**, 417–424.

Received on February 20, 1997; revised on June 23, 1997