Biochimica et Biophysica Acta 1843 (2014) 1551–1558

Contents lists available at ScienceDirect

Biochimica et Biophysica Acta

journal homepage: www.elsevier.com/locate/bbamcr

Curli biogenesis: Order out of disorder☆

Margery L. Evans, Matthew R. Chapman *

Department of Molecular, Cellular and Developmental Biology, University of Michigan, 830 North University, Ann Arbor, MI 48109, USA

ARTICLE INFO

Article history:
Received 21 August 2013
Received in revised form 18 September 2013
Accepted 19 September 2013
Available online 27 September 2013

Keywords:
Curli
Functional amyloid
Aggregate
Biofilm
Nucleation–precipitation
Type VIII secretion

ABSTRACT

Many bacteria assemble extracellular amyloid fibers on their cell surface. Secretion of proteins across membranes and the assembly of complex macromolecular structures must be highly coordinated to avoid the accumulation of potentially toxic intracellular protein aggregates. Extracellular amyloid fiber assembly poses an even greater threat to cellular health due to the highly aggregative nature of amyloids and the inherent toxicity of amyloid assembly intermediates. Therefore, temporal and spatial control of amyloid protein secretion is paramount. The biogenesis and assembly of the extracellular bacterial amyloid curli is an ideal system for studying how bacteria cope with the many challenges of controlled and ordered amyloid assembly. Here, we review the recent progress in the curli field that has made curli biogenesis one of the best-understood functional amyloid assembly pathways. This article is part of a Special Issue entitled: Protein trafficking and secretion in bacteria. Guest Editors: Anastassios Economou and Ross Dalbey.

1. Curli are functional amyloids

Amyloids have been intensely studied for decades and are hallmarks of many human illnesses including Alzheimer's, Parkinson's and Huntington's diseases [1–3]. More recently, a new class of amyloids–the so-called 'functional' amyloids–has been described, and new members of this class are rapidly being discovered. Functional amyloids are now believed to be ubiquitous and they perform an enormous diversity of biological functions [4–8]. Numerous functional amyloids are produced by a variety of microbes with the majority of them playing roles in adhesion to surfaces and structural integrity during the establishment and persistence of biofilm communities [9].

Curli, the first identified functional amyloid, are extracellular protein fibers produced by many enteric bacteria including *Escherichia coli* and *Salmonella* species (Fig. 1A) [10–13]. Curli are the major proteinaceous component of *E. coli* biofilms and are important for surface colonization and interacting with host factors and the host immune system [10,14–21]. Curli production is easily scored in the lab by plating cells on agar plates containing the amyloid binding dye Congo red [17,22]. Like all amyloids, the major curli subunit CsgA is capable of self-polymerizing *in vitro* into β-sheet-rich amyloid fibers that bind to the amyloid specific dye Thioflavin T (ThT) and can be visualized using transmission electron microscopy (TEM) [4,23,24]. *In vitro* assembled CsgA amyloid fibers are indistinguishable from fibers formed *in vivo* [23].

The ability of curli to act as a strong scaffolding agent in biofilm formation stems from properties inherent to all amyloids. Curli are highly stable 6–12 nm wide non-branching fibers (Fig. 1A) that are resistant to degradation by proteases and denaturation by detergents [4]. Pretreatment with a strong denaturant such as formic acid or hexafluoroisopropanol is required to depolymerize curli fibers so that monomers of the major subunit protein, CsgA, can be resolved on an SDS-PAGE gel [4]. CsgA amyloids, like other amyloids, are β-sheet rich and assemble into a highly stable cross-β structure stabilized in large part by tight "steric zipper" interactions between side-chains on adjacent β-sheets [4,25–27]. The amyloid characteristics of curli fibers are clearly important for their biological function and afford many useful techniques for rapid *in vivo* and *in vitro* study of curli biogenesis, biofilm formation and integrity, and amyloid assembly [28,29].

2. Curli expression is highly coordinated

Curli gene expression is a highly regulated and coordinated event both on the cellular level and within a biofilm community. Curli are the major protein component of *E. coli* biofilms yet the production of curli within a biofilm is restricted to a distinct subpopulation [14,30–33]. Expression is controlled by several environmental signals and chemical gradients including temperature, osmolarity and oxygen [34–36]. Within a rugose, or rough, colony biofilm (Fig. 1B) curliated cells localize to the air-colony interface [30,33]. This bimodal population development can be triggered by oxidative stress, and in turn, rugose biofilms are

☆ This article is part of a Special Issue entitled: Protein trafficking and secretion in bacteria. Guest Editors: Anastassios Economou and Ross Dalbey.

* Corresponding author. Tel.: +1 734 764 7592; fax: +1 734 647 0884.
E-mail address: chapmanm@umich.edu (M.R. Chapman).

0167-4889/$ – see front matter © 2013 Elsevier B.V. All rights reserved.
http://dx.doi.org/10.1016/j.bbamcr.2013.09.010

protected from oxidative damage resulting from exposure to hydrogen peroxide [30]. Bacteria within *Salmonella enterica* curli- and cellulose-dependent rugose colonies are also protected from desiccation and are more resistant to sodium hypochlorite treatment (or bleach) when compared to non-curliated colonies or planktonic cells, respectively [37]. Successful biofilm formation requires spatial and temporal regulation of curli assembly, which relies on elaborate coordination of gene expression and protein production (Table 1).

The curli specific genes (*csg*) are found in two divergently transcribed operons, the intergenic region of which is the 7th largest in *E. coli* and subject to extensive regulation [17,38,39]. The *csgDEFG* promoter is recognized as one of the most complexly regulated promoters in *E. coli* [40]. Curli are primarily expressed during stationary phase and at low temperatures (below 30 °C), although some clinical isolates can express curli at 37 °C [10,11,35,41]. Both curli promoters are regulated by the stationary phase alternative sigma factor σ^S, which is assisted by the thermosensitive protein Crl (Table 1) [10,41,42]. Stationary phase expression of the *csgDEFG* promoter is likewise positively regulated by the stationary phase transcription factor MlrA (Table 1) [43,44]. Curli expression is also internally regulated by the first gene product of the *csgDEFG* operon: CsgD (Table 1). CsgD is a member of the FixJ/LuxR family of transcriptional regulators that coordinates the expression of multiple biofilm components including curli and cellulose, while repressing expression of flagellar genes [17,45–47]. CsgD activity and stability is modulated by phosphorylation of an N-terminal aspartic acid residue (DNA binding is decreased by *in vitro* phosphorylation) [48]. Several additional transcriptional regulators modulate expression of the *csgDEFG* promoter: the catabolite repressor/activator protein Cra, the cAMP receptor protein CRP, and the recently identified protein RcdA (Table 1) [43,44,49–51]. At least two known two-component systems negatively regulate the *csgDEFG* operon (CpxA/R and RcsA/B) and one that positively does (OmpR/EnvZ) (Table 1) [52–57]. The two global DNA organization protein complexes IHF and H-NS impact curli gene expression inversely: IHF contributes to promote curli gene expression while H-NS represses curli expression in *E. coli* (Table 1) [58–60]. Both positive and negative regulation involves simultaneous binding of regulatory proteins as well as competitive binding between positive and negative regulators. For instance, H-NS and IHF act in competition for binding upstream of the *csgDEFG* promoter while CpxR and H-NS can bind simultaneously to negatively regulate the *csgDEFG* promoter and OmpR and RstA can bind simultaneously with IHF to positively regulate the *csgD* promoter [59]. Finally, the *csgDEFG* transcript is also subject to negative post-transcriptional regulation by five small regulatory RNAs: OmrA, OmrB, McaS, GcvB and RprA (Table 1) [61–64]. The small RNAs ArcZ and SdsR also positively regulate *csgD* in *S. enterica*, but whether this is a direct effect has yet to be determined (Table 1) [65]. For a more comprehensive review of the role of small RNAs in regulating the transition between biofilm and sessile growth see Mika and Hengge, 2013 [66].

3. Building a curli fiber on the cell surface

3.1. CsgA amyloid assembly by design

A striking aspect of curli biogenesis is that, despite CsgA's propensity to aggregate, amyloid formation is so faithfully restricted to the cell surface. The major and minor curli subunits, CsgA and CsgB, respectively, are encoded by the *csgBAC* operon [17,38,67]. CsgA is secreted across the outer membrane as an unstructured soluble peptide (Fig. 2) [68]. Soluble CsgA is then templated into an amyloid on the cell surface by the nucleator and minor curli subunit CsgB (Fig. 2) [69,70]. This mechanism of secretion is termed nucleation–precipitation or Type VIII secretion [71]. A third, and less well understood, gene *csgC* is located downstream of *csgA*. CsgC is a small periplasmic protein that is proposed to play a role in subunit secretion [68,72]. CsgC is a β-sheet rich protein with an immunoglobulin-like fold and a conserved CXC motif that shares a high degree of structural similarity with the N-terminus of the redox-active DsbD [72]. Whether the other curli proteins are redox substrates of CsgC is still unclear; the curli pore protein CsgG, however, is an attractive substrate candidate as it is the only other curli protein that contains a solvent exposed cysteine residue [72]. Additionally, the presence of CsgC renders the *csgBA* mutant more sensitive to bile salts compared to the *csgBAC* mutant suggesting that CsgC may keep the pore open or ungated [72]. Furthermore, an *S.* Enteritidis *csgC* mutant produces wider curli fibers (~20 nm) suggesting that CsgC may also function in CsgA folding and curli fiber structure [68]. The *csgE, csgF* and *csgG* genes encode the proteins that comprise the unique outermembrane curli secretion apparatus (Fig. 2). CsgG assembles into a pore-like structure in the outermembrane while CsgE and CsgF play chaperone-like functions assisting in secretion and cell surface attachment of the curli fibers (Fig. 2) [73–75]. CsgD is a positive regulator that promotes biofilm formation by positively regulating curli and cellulose production (Table 1) [17,45–47].

CsgA is the major subunit of the curli fiber and is secreted across the outermembrane as an unstructured and soluble monomer (Fig. 2) [4,17,68,76,77]. The mature CsgA protein is comprised of an N-terminal 22 amino acids (N-term22) required for outermembrane secretion, and a C-terminal amyloid core domain (Fig. 3A,B) [25,26,75]. The amyloid core of CsgA is composed of five imperfect repeating units: R1 through R5 (Fig. 3A,B) [23,77]. Each repeating unit contains a Ser-X_5-Gln-X-Gly-X-Gly-Asn-X-Ala-X_3-Gln motif and forms a β-sheet-turn-β-sheet

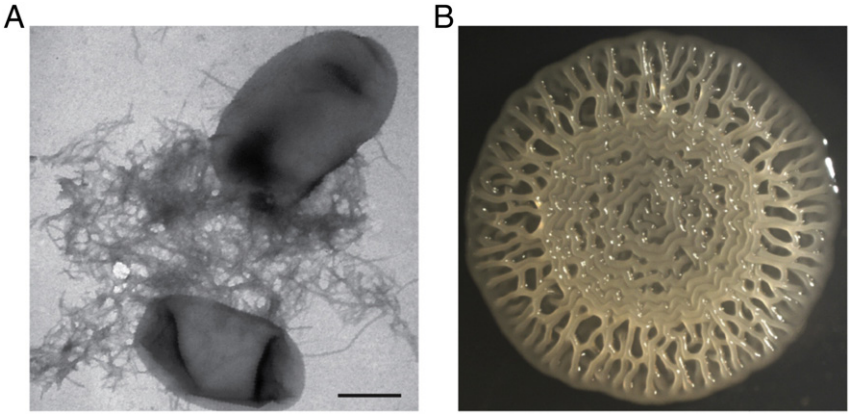

Fig. 1. Curli production contributes to *E. coli* biofilms. A. *E. coli* k-12 strain BW25113 grown on a low salt agar plate at 26 °C produce cell surface associated curli fibers that are visible by transmission electron microscopy. Scale bar is 500 nm. B. The uropathogenic *E. coli* strain UTI89 develops a complex rugose colony morphology that is dependent on curli.

Table 1
Direct regulators of the curli operons.

Regulator	P_{csgBAC}	$P_{csgDEFG}$	Evidence	Reference(s)
Transcriptional regulators				
RpoS	+	+	Expression analysis, consensus binding site	[42,60,102,103]
Crl	+	+	Interacts with RpoS and stabilizes binding to *csg* promoters	[41,42]
CsgD	+	+	Expression analysis, consensus binding site	[17,47]
MlrA		+	Expression analysis, consensus binding site	[43,44]
Cra (FruR)		+	Expression analysis, consensus binding site	[49]
Crp		+	Expression analysis	[51]
RcdA		+	Expression analysis, consensus binding site	[50]
DNA modifying enzymes				
IHF		+	Expression analysis, consensus binding site	[58,59]
H-NS		−/+ (*E. coli*/*S. typhimurium*)	Expression analysis, consensus binding site	[59,60,102]/[58]
Two-component systems				
CpxA/R	−	−	Expression analysis, consensus binding site	[52,53,59]
EnvZ/OmpR		+	Expression analysis, consensus binding site	[53,55,57–59,104]
RcsA/B	−	−	Expression analysis	[53,54,105]
RstB/A		−/+ (basic/acidic conditions)	Expression analysis, consensus binding site	[59,106]
ArcA/B		+	Expression analysis, consensus binding site	[107]
BasS/R		+	Expression analysis, consensus binding site	[108]
Small regulatory RNAs				
OmrA/B		−	Antisense binding to 5′UTR	[61]
McaS		−	Antisense binding to 5′UTR	[62,64]
GcvB		−	Antisense binding to 5′UTR	[62]
RprA		−	Antisense binding to 5′UTR	[62,63]
ArcZ		+	Expression analysis post-transcriptional regulation	[65]
SdsR		+	Expression analysis, transcriptional regulation	[65]

Regulators of the *csgBAC* and *csgDEFG* promoters. Positive and negative regulation of each promoter is indicated by "+" or "−", repectively.

secondary structure with an overall β-helical, cross-β structure (Fig. 3A,B) [25,26]. The arrangement of the repeating units in the cross-β structure aligns the Gln and Asn residues in each repeating unit in stacks that stabilize the amyloid fold (Fig. 3A) [78]. It is also worth noting that the placement and stacking of the Gly and Ala residues in the consensus sequence is highly conserved in Gammaproteobacteria and likely play an important role in amyloid assembly [13].

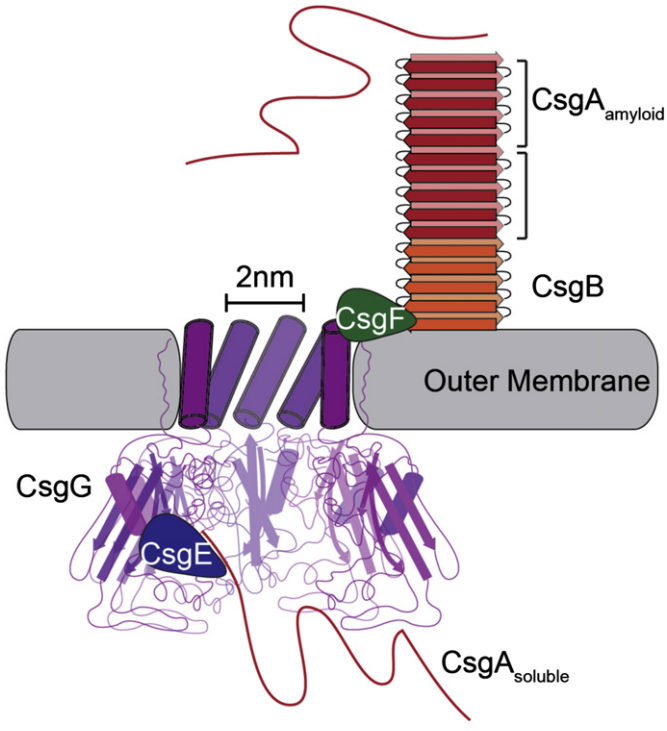

Fig. 2. The curli biogenesis system possesses a unique outer membrane secretion apparatus. The CsgG pore is composed of eight subunits (the front three have been removed to improve clarity) with a 2 nm wide central pore. Both CsgE and CsgF interact directly with the CsgG pore. CsgE is periplasmic and required for directing soluble CsgA to the CsgG pore for secretion while CsgF is surface exposed and contributes to the assembly of CsgB into a surface associated, amyloid-templating conformation. Once outside the cell, CsgA interacts with CsgB and assembles into an amyloid fiber.

Purified CsgA self-assembles into amyloid fibers that bind ThT and can be visualized by transmission electron microscopy (TEM) *in vitro*. CsgA polymerization *in vitro* is very robust: CsgA polymerizes into indistinguishable amyloid fibers over large pH (3.0–9.0) and ionic (0–500 mM NaCl) ranges [24]. CsgA polymerization is concentration dependent and can be divided into three phases: a lag phase, an elongation phase and a stationary phase. At CsgA concentrations between 5 and 30 μM, the lag phase is approximately 2 h [23,24]. During the lag and elongation phases, a CsgA folding intermediate that is conserved in most amyloid-forming proteins can be detected with a conformation-specific antibody [23,79]. Although purified CsgA readily forms amyloid *in vitro*, CsgA amyloid formation *in vivo* requires the nucleator protein CsgB [69,70]. CsgB nucleated polymerization of CsgA can be recapitulated *in vitro* in a process called "seeding". When added to soluble CsgA, preformed CsgB or CsgA fibers (also referred to as seeds) accelerate the polymerization of CsgA by eliminating the lag phase [78]. Interestingly, curli subunits from closely related species exhibit cross-seeding promiscuity. Curli subunits from *E. coli* and the closely related bacteria *Citrobacter koseri* and *S. enterica* are capable of cross-seeding both *in vivo* and *in vitro* [80].

The molecular features of both CsgA and CsgB that contribute to amyloid formation and nucleation have been thoroughly dissected. The repeating units of CsgA have distinct characteristics that either promote or temper amyloid formation (Fig. 3AB) [78,81]. Repeating units R1, R3, and R5 are amyloidogenic, while R2 and R4 are non-amyloidogenic: synthetic peptides of R1, R3 and R5 rapidly assemble into amyloid fibers *in vitro* while R2 and R4 do not [23,82]. Furthermore, CsgA ΔR1 and ΔR5 do not assemble into curli fibers *in vivo* and cannot be seeded by either CsgA or CsgB *in vitro* [82]. R1 and R5 are therefore not only important for amyloid assembly, but are also the domains that mediate CsgA–CsgA and CsgA–CsgB interactions. The Gln and Asn residues in R1 and R5 (at positions 49, 54, 139 and 144) are critical for amyloid formation (Fig. 3A) [78]. Mutation of the Gln and Asn residues in R1 and R5 results in a CsgA variant, aptly named $CsgA_{slowgo}$, that polymerizes with a

significantly extended (~100× longer) lag phase compare to WT CsgA [23,78]. While R1, R3 and R5 synthetic peptides all polymerize into amyloids *in vitro*, R3 polymerizes more slowly [82]. R1 and R5 are interchangeable with one another (R12341 and R52345 CsgA chimeras produce WT curli *in vivo*), while replacement of either R1 or R5 with R3 (R32345 or R12343) results in little to no curli [82].

Certain residues in the CsgA repeating units appear to temper or slow CsgA aggregation into amyloid [81]. Asp residues at positions 91 and 104 within R3 have been identified as "gatekeeper" residues that reduce the amyloidogenicity of R3 (Fig. 3A) [81]. Mutations to either of these Asp residues in such a way that the peptide more closely resembles R1 or R5 restore curli assembly of the R12343 chimera *in vivo* and polymerization *in vitro* [81]. Conversely, substitution of Asn136 and His149 within R5 for Asp, thus rendering R5 more similar to R3, abolishes curli assembly *in vivo* [81]. The non-amyloidogenic repeating units R2 and R4 also contain "gatekeeper" residues (Fig. 3A). Mutating Gly78/Asp80/Gly82 in R2 and Gly123/Asp127 in R4 to the corresponding amino acids in R1 and R5, respectively, restored curli assembly to the CsgA ΔR1 and ΔR5 truncations *in vivo* [81]. Mutation of all of the "gatekeeper" residues in R2, R3 and R4 to the corresponding residues in R1 or R5 in full-length CsgA results in a variant (CsgA*) that polymerizes without a lag phase *in vitro* [81]. Furthermore, CsgA* is capable of forming curli *in vivo* in a CsgB/CsgF-independent manner and exhibits a toxic effect when overexpressed [81]. These "gatekeeper" residues enable a level of intramolecular control over CsgA amyloid assembly that is important in avoiding amyloid-associated cytotoxicity.

3.2. Amyloid assembly is nucleation-dependent in vivo

In addition to keeping CsgA polymerization in check with intramolecular "gatekeeping" residues, curli assembly is also controlled by dividing the tasks of nucleation and polymerization between two proteins, CsgB and CsgA, respectively. CsgB is dispensable for polymerization of CsgA *in vitro* but is required for curli assembly on the cell surface *in vivo* [4,23,69,70,82]. In the absence of the surface-associated CsgB, CsgA is secreted away from the cell and remains SDS-soluble [69,83,84]. However, soluble CsgA either supplemented exogenously or from a nearby *csgB* mutant donor cell can be engaged on the surface of cells presenting CsgB in a process referred to as interbacterial complementation (Fig. 4) [4,69,82]. Donor and recipient strains streaked on an agar plate as far apart as 3 mm can still participate in interbacterial

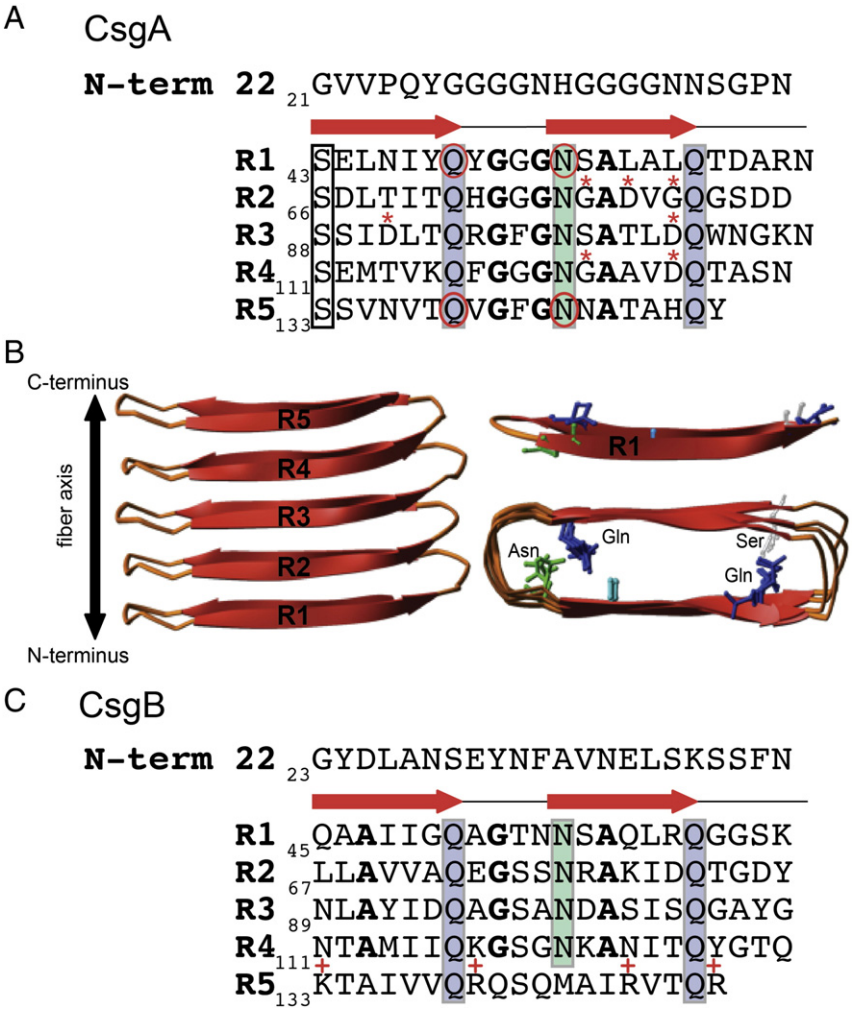

Fig. 3. The molecular details of CsgA and CsgB. A. The mature CsgA protein is composed of an N-terminal 22 amino acids and 5 amyloid repeating units, each with a β-sheet-turn-β-sheet (indicated by the red arrows). Ser, Gln and Asn residues (boxes) in each repeating unit align in stacks that stabilize the amyloid conformation. The Gln residues at positions 49 and 139 and the Asn residues at positions 54 and 144 (circled) are essential for amyloid formation. Repeating unites R2, R3 and R4 contain "gatekeeper" residues (*) that temper amyloid formation. The conserved glycine and alanine residues are indicated in bold font. B. A cartoon representation of the predicted structure of CsgA shows the five repeating units assemble into a β-helical, cross-β structure (side view, left). The Gln, Asn and Ser residues of R1 (upper right) are shown as sticks and the overhead view of CsgA (lower right) shows the alignment of these residues within the predicted structure. C. The mature CsgB protein is composed of an N-terminal 22 amino acids and 5 amyloid repeating units with similarly conserved Gln and Asn stacks (boxes). The conserved glycine and alanine residues in R1–R4 are indicated in bold font. Repeating unit 5 lacks one of the Asn repeats, but instead contains four charged residues (indicated by +) that may be important for tethering CsgB to the cell surface *via* R5.

complementation, owing to the incredible efficiency of the nucleation–precipitation mechanism of curli assembly (Fig. 4) [69].

Like CsgA, CsgB is a glutamine and asparagine-rich protein comprised of a SEC secretion signal sequence, an N-terminal domain and a core domain (Fig. 3C). CsgB shares 30% sequence identity with CsgA and has an amyloid-like core similar to CsgA [70,85]. Each of the first four repeating units of CsgB contains an Ala-X_3-Gln-X-Gly-X_2-Asn-X-Ala-X_3-Gln motif similar to the repeat motif of CsgA. Repeating unit 5, however, lacks several of these residues and instead contains four positively charged amino acids that are absent in the other CsgB repeating units: Lys133, Arg140, Arg147 and Arg151 (Fig. 3C). Deletion of repeating unit 5 results in a truncated version of CsgB ($CsgB_{trunc}$) that is no longer cell surface associated [86]. Moreover, $CsgB_{trunc}$ self-assembles into amyloid fibers *in vitro* and can seed the polymerization of CsgA consistent with the hypothesis that CsgB also assembles into an amyloid core [70]. Repeating units 1–3 appear to be largely dispensable for *in vitro* polymerization and localization, leaving R4 and R5 of primary importance for localization and nucleation of CsgA [86]. However, it is also possible that some of the CsgB repeating units function redundantly. These observations suggest a model where CsgB associates with the cell membrane *via* the fifth repeating unit while R1–R4 assume an amyloid fold that can template soluble CsgA into amyloid fibers. How CsgB is associated with the outermembrane is still largely unknown; however, CsgB localization requires at least one component of the curli secretion apparatus, CsgF [74].

3.3. Curli are secreted by a dedicated outermembrane secretion apparatus

The curli system possesses a unique outermembrane secretion apparatus comprised of at least three proteins: CsgG, CsgE and CsgF (Fig. 2) [4,17,84]. CsgG is a lipoprotein that spans the outermembrane and assembles into an oligomeric annular-shaped structure with an inner diameter of approximately 2 nm [72,75,84]. High-resolution electron microscopy and modeling studies predict that the transmembrane domain of CsgG is alpha-helical while the periplasmic domain is predominantly random coil and β-sheet rich with a single α-helix (Fig. 2) [72]. Overexpression of CsgG results in increased sensitivity to the small antibiotic erythromycin (740 Da) but not the larger vancomycin (1440 Da) suggesting that CsgG has a pore-like activity [75]. Overexpression of CsgE complements the erythromycin sensitivity phenotype of CsgG overexpressing strains [31,73]. CsgE may therefore act, in part, by gating CsgG to prevent the influx of environmental chemicals and molecules through the pore.

The CsgG oligomers are not uniformly distributed around the cell but are instead spatially restricted to discrete regions of the cell that co-localize with what appears to be the primary curli fiber attachment site [31]. The mechanism of CsgG localization, however, and how the curli secretion apparatus is spatially localized to discrete spots around the cell remains unknown. Because subcellular localization is important for many biological functions, including macromolecular organelle assembly in bacteria, it is not unreasonable to hypothesize that spatial restriction of the curli secretion apparatus is also important for curli assembly [87,88]. Diffusion of protein subunits into the environment is a significant challenge of the nucleation–precipitation/Type VIII secretion system [71,89]. Localized secretion of CsgA may lead to high local concentrations that promote efficient curli formation on the cell surface. Interestingly, CsgA and CsgB are both required for stable oligomerization of CsgG suggesting that either subunit secretion or active curli polymerization on the cell surface somehow facilitates stable pore assembly [31].

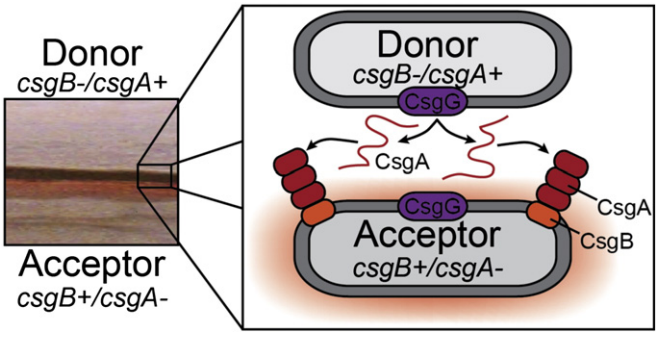

Fig. 4. Curli subunit sharing between adjacent cells, or interbacterial complementation, is made possible by the nucleation–precipitation mechanism of curli fiber assembly. A *csgB* mutant (or *csgA* + donor strain) secretes soluble CsgA into the extracellular milieu. CsgA from a *csgB* mutant can polymerize on a *csgA* mutant (or *csgB* + acceptor) that is presenting CsgB on the cell surface when grown in close proximity to one another on a plate. The left panel shows a *csgB* mutant streaked near a *csgA* mutant, and on the edge of the CsgB-expressing streak there is a Congo red staining region.

Stable oligomerization of CsgG is dependent on both of the major and minor curli subunits, CsgA and CsgB, as well as the accessory proteins CsgE and CsgF [31]. Conversely, CsgG is required for the stability and secretion of CsgA, CsgB and CsgF across the outermembrane [74,84]. CsgG interacts with CsgE and CsgF as well as fusion proteins in which the N-term22 of CsgA has been fused to the N-terminus of a substrate protein [75]. CsgF is extracellular and interacts with CsgG on the cell surface where it stabilizes and localizes CsgB to the cell-surface [74]. In the absence of CsgF, both CsgB and CsgA remain predominately SDS-soluble and are secreted away from the cell [74]. Overexpression of CsgB in a *csgF* mutant restores the cell-surface localization of CsgB but it does not restore WT cell-surface associated curli fibers [74]. CsgF therefore may play a more important role in remodeling CsgB into an amyloid-templating conformation rather than actually anchoring CsgA fibers.

Ensuring that curli amyloid assembly occurs at the correct time and place is a feat that is at least in part achieved by molecular chaperones [73,90]. Nenninger et al. described CsgF as having chaperone-like activity: CsgF appears to assist in directing the assembly of CsgA into an amyloid on the cell surface [74]. CsgE, on the other hand, functions earlier in the curli biogenesis pathway by inhibiting premature amyloid formation within the periplasm, but also by directing CsgA subunits to CsgG for secretion [73]. CsgE inhibits CsgA amyloid formation and interacts with CsgA amyloid fibers *in vitro* [73,91]. Two lines of evidence using fusion protein constructs with the N-terminal domain of CsgA implicate the N-terminal 22 amino acids of CsgA as the interacting domain with both CsgE and CsgG [73,75]. First, the large periplasmic protein PhoA coimmunoprecipitated with CsgG when PhoA was fused to the N-terminal 42 amino acids of CsgA (including the SEC secretion signal sequence) [75]. Second, expression of CsgE specifically permits the secretion of the second domain of PapD or full-length CpxP only when the C-terminus is fused to the N-terminal 22 amino acids of CsgA [73]. However, overexpression of CsgG results in unchecked secretion indicating that secretion through CsgG doesn't necessarily require CsgE [73,75]. Together, these results suggest a coordinated chaperone/delivery and secretion mechanism. CsgE putatively has three functions: 1) bind to CsgG and gate the outer membrane secretion pore, 2) bind to and inhibit amyloid formation of curli subunits within the periplasm, and 3) deliver these subunits to CsgG for secretion.

4. The curli system can be exploited for studying other amyloids

Other bacteria may have also adapted the CsgG-like amyloid secretion pore. The *Caulobacter crescentus* holdfast protein HfaA shares the amyloid-like characteristics of being resistant to heat and SDS denaturation [92]. Like CsgA, the secretion and stability of HfaA depends on an outermembrane lipoprotein, HfaB, which shares 31% identity and 42% similarity with CsgG [93]. It is attractive to hypothesize that the curli secretion mechanism is representative of a distinct class of apparatuses for secreting amyloid subunits across membranes.

The unique curli secretion apparatus may be ideally suited for transporting generic unfolded amyloid proteins across the outer membrane. CsgA is unstructured upon secretion and the CsgG pore, only about 2 nm (or 20 Å) wide, is not large enough to secrete CsgA in its folded conformation, suggesting that CsgA is maintained in an unstructured and soluble conformation prior to secretion [68,75,77]. The N-terminal 22 amino acids of the mature CsgA not only direct its secretion across the outer membrane, but when fused to unrelated amyloid proteins, can also direct their secretion [29,73,75,94]. *E. coli* expressing fusions of unrelated amyloids with the N-terminal domain of CsgA produce Congo red binding colonies on agar plates and SDS-insoluble fibers [29,94]. Harnessing the curli secretion system to assemble heterologous amyloids on the bacterial cell surface may pave the way for new high throughput screening methods for modulators of amyloid assembly.

5. Folding intermediates can be probed using antibodies and small molecules

The discovery of conformation specific antibodies has contributed immensely to our understanding of amyloid assembly [79,95,96]. We now have the tools to detect intermediate oligomeric and fibrillar species during the amyloid assembly process. The conformation-specific antibody A11 was raised against an oligomeric intermediate of Aβ, the amyloid protein associated with Alzheimer's disease, and reacts with the oligomeric intermediates of many other disease-associated amyloids [79]. CsgA is also transiently recognized by A11 *in vitro* indicating that CsgA functional amyloid formation proceeds by the same mechanism of assembly as disease-associated amyloids *in vitro* [23]. Antibodies have also been raised that specifically recognize the amyloid fiber conformation and also broadly react with unrelated amyloid fibers [95,96]. Conformation specific antibodies have motivated the development of a new class of antibodies, gammabodies (for grafted *am*yloid *m*otif anti*bodies*), that not only react specifically with the fiber conformation, but also have a high degree of specificity for protein sequence [97,98]. Gammabodies have been demonstrated to act in distinct mechanisms, depending on the grafted sequence, to inhibit amyloid formation of several disease-associated amyloids [97]. Gammabodies have been engineered against each repeating unit of CsgA and have been shown to exhibit the same sequence and conformation specificity as gammabodies designed to interact with disease-associated amyloids [91,98]. It has yet to be determined if similar inhibitory interactions are possible between gammabodies and functional amyloids.

Peptidomimetic compounds are also attractive candidates for modulating protein–protein interactions and amyloid formation. A screen of compounds originally designed to inhibit the elaboration of *E. coli* P pili yielded a set of small molecules capable of inhibiting amyloid formation of Aβ [99,100]. These compounds have since been subject to extensive chemical modification and assayed for biological activity against several amyloid substrates including curli [28,91,101]. This extensive library of 2-pyridone compounds, or curlicides, exhibit varying degrees of CsgA amyloid inhibition and curli-dependent biofilm inhibition [28,91,101]. Interestingly, the compound FN075 inhibits both Aβ and CsgA amyloid formation but accelerates amyloid formation of the Parkinson's Disease-associated protein α-synuclein [101]. FN075 promotes the oligomerization of both CsgA and α-synuclein, however, the α-synuclein oligomers are competent for amyloid formation (on-pathway) while the CsgA oligomers are not (off-pathway) [101]. These provocative results suggest that the 2-pyridone compounds may be used as tools for probing and distinguishing between on- and off-pathway protein folding intermediates. Andersson et al. have now identified other 2-pyridone compounds that are capable of accelerating CsgA polymerization [91]. Further analysis of curli assembly in the presence of these curlicide and accelerator compounds may be instrumental in dissecting early stages of functional amyloid assembly.

6. Conclusions

The amyloid field has undergone a sea change since curli were characterized as an amyloid and the functional amyloid field was born. Curli biology is ideal for studying protein secretion and folding in the context of bacterial community behavior and, remarkably, can be amended for studying of protein folding and misfolding in the context of many human diseases. Elaborate mechanisms are in place to ensure that curli are assembled only at the right time, at the right place and with remarkable efficiency to overcome large diffusion rates inherent in a nucleation–precipitation model. It seems only natural that the unique properties of curli fibers are accompanied by an equally unique biogenesis system.

Acknowledgements

We would like to thank W. H. DePas and D. A. Hufnagel for critical reading of this manuscript and W. H. DePas for contributing the rugose colony image in Fig. 1B. This work was supported by the National Institutes of Health (AI073847 to M. R. C.), the University of Michigan Cellular Biotechnology Training Program (NIH NRSA T32 GM008353) and the University of Michigan 2013–2014 Rackham Graduate School Predoctoral Fellowship.

References

[1] J. Hardy, D.J. Selkoe, The amyloid hypothesis of Alzheimer's disease: progress and problems on the road to therapeutics, Science 297 (2002) 353–356.
[2] L. Breydo, J.W. Wu, V.N. Uversky, Alpha-synuclein misfolding and Parkinson's disease, Biochim. Biophys. Acta 1822 (2012) 261–285.
[3] C. Zuccato, M. Valenza, E. Cattaneo, Molecular mechanisms and potential therapeutical targets in Huntington's disease, Physiol. Rev. 90 (2010) 905–981.
[4] M.R. Chapman, L.S. Robinson, J.S. Pinkner, R. Roth, J. Heuser, M. Hammar, S. Normark, S.J. Hultgren, Role of *Escherichia coli* curli operons in directing amyloid fiber formation, Science 295 (2002) 851–855.
[5] D. Romero, C. Aguilar, R. Losick, R. Kolter, Amyloid fibers provide structural integrity to *Bacillus subtilis* biofilms, Proc. Natl. Acad. Sci. U. S. A. 107 (2010) 2230–2234.
[6] T. Scheibel, A.S. Kowal, J.D. Bloom, S.L. Lindquist, Bidirectional amyloid fiber growth for a yeast prion determinant, Curr. Biol. 11 (2001) 366–369.
[7] D.M. Fowler, A.V. Koulov, C. Alory-Jost, M.S. Marks, W.E. Balch, J.W. Kelly, Functional amyloid formation within mammalian tissue, PLoS Biol. 4 (2006) e6.
[8] P. Larsen, J.L. Nielsen, M.S. Dueholm, R. Wetzel, D. Otzen, P.H. Nielsen, Amyloid adhesins are abundant in natural biofilms, Environ. Microbiol. 9 (2007) 3077–3090.
[9] L.P. Blanco, M.L. Evans, D.R. Smith, M.P. Badtke, M.R. Chapman, Diversity, biogenesis and function of microbial amyloids, Trends Microbiol. 20 (2012) 66–73.
[10] A. Olsen, A. Jonsson, S. Normark, Fibronectin binding mediated by a novel class of surface organelles on *Escherichia coli*, Nature 338 (1989) 652–655.
[11] X. Zogaj, W. Bokranz, M. Nimtz, U. Romling, Production of cellulose and curli fimbriae by members of the family Enterobacteriaceae isolated from the human gastrointestinal tract, Infect. Immun. 71 (2003) 4151–4158.
[12] X. Zogaj, M. Nimtz, M. Rohde, W. Bokranz, U. Romling, The multicellular morphotypes of *Salmonella* typhimurium and *Escherichia coli* produce cellulose as the second component of the extracellular matrix, Mol. Microbiol. 39 (2001) 1452–1463.
[13] M.S. Dueholm, M. Albertsen, D. Otzen, P.H. Nielsen, Curli functional amyloid systems are phylogenetically widespread and display large diversity in operon and protein structure, PLoS One 7 (2012) e51274.
[14] O.A. McCrate, X. Zhou, C. Reichhardt, L. Cegelski, Sum of the Parts: Composition and Architecture of the Bacterial Extracellular Matrix, J. Mol. Biol. (2013) (in press, PubMed ID: 23827139).
[15] D.M. Pawar, M.L. Rossman, J. Chen, Role of curli fimbriae in mediating the cells of enterohaemorrhagic *Escherichia coli* to attach to abiotic surfaces, J. Appl. Microbiol. 99 (2005) 418–425.
[16] R.R. Boyer, S.S. Sumner, R.C. Williams, M.D. Pierson, D.L. Popham, K.E. Kniel, Influence of curli expression by *Escherichia coli* 0157:H7 on the cell's overall hydrophobicity, charge, and ability to attach to lettuce, J. Food Prot. 70 (2007) 1339–1345.
[17] M. Hammar, A. Arnqvist, Z. Bian, A. Olsen, S. Normark, 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 (1995) 661–670.
[18] S.K. Collinson, P.C. Doig, J.L. Doran, S. Clouthier, T.J. Trust, W.W. Kay, Thin, aggregative fimbriae mediate binding of *Salmonella enteritidis* to fibronectin, J. Bacteriol. 175 (1993) 12–18.
[19] A. Olsen, M.J. Wick, M. Morgelin, L. Bjorck, Curli, fibrous surface proteins of *Escherichia coli*, interact with major histocompatibility complex class I molecules, Infect. Immun. 66 (1998) 944–949.
[20] Y. Kai-Larsen, P. Luthje, M. Chromek, V. Peters, X. Wang, A. Holm, L. Kadas, K.O. Hedlund, J. Johansson, M.R. Chapman, S.H. Jacobson, U. Romling, B. Agerberth, A. Brauner, Uropathogenic *Escherichia coli* modulates immune responses and its

curli fimbriae interact with the antimicrobial peptide LL-37, PLoS Pathog. 6 (2010) e1001010.
[21] Z. Bian, A. Brauner, Y. Li, S. Normark, Expression of and cytokine activation by *Escherichia coli* curli fibers in human sepsis, J. Infect. Dis. 181 (2000) 602–612.
[22] M.R. Nilsson, Techniques to study amyloid fibril formation *in vitro*, Methods 34 (2004) 151–160.
[23] X. Wang, D.R. Smith, J.W. Jones, M.R. Chapman, *In vitro* polymerization of a functional *Escherichia coli* amyloid protein, J. Biol. Chem. 282 (2007) 3713–3719.
[24] M.S. Dueholm, S.B. Nielsen, K.L. Hein, P. Nissen, M. Chapman, G. Christiansen, P.H. Nielsen, D.E. Otzen, Fibrillation of the major curli subunit CsgA under a wide range of conditions implies a robust design of aggregation, Biochemistry 50 (2011) 8281–8290.
[25] S.K. Collinson, J.M. Parker, R.S. Hodges, W.W. Kay, Structural predictions of AgfA, the insoluble fimbrial subunit of *Salmonella* thin aggregative fimbriae, J. Mol. Biol. 290 (1999) 741–756.
[26] F. Shewmaker, R.P. McGlinchey, K.R. Thurber, P. McPhie, F. Dyda, R. Tycko, R.B. Wickner, The functional curli amyloid is not based on in-register parallel beta-sheet structure, J. Biol. Chem. 284 (2009) 25065–25076.
[27] R. Nelson, M.R. Sawaya, M. Balbirnie, A.O. Madsen, C. Riekel, R. Grothe, D. Eisenberg, Structure of the cross-beta spine of amyloid-like fibrils, Nature 435 (2005) 773–778.
[28] L. Cegelski, J.S. Pinkner, N.D. Hammer, C.K. Cusumano, C.S. Hung, E. Chorell, V. Aberg, J.N. Walker, P.C. Seed, F. Almqvist, M.R. Chapman, S.J. Hultgren, Small-molecule inhibitors target *Escherichia coli* amyloid biogenesis and biofilm formation, Nat. Chem. Biol. 5 (2009) 913–919.
[29] V. Sivanathan, A. Hochschild, Generating extracellular amyloid aggregates using *E. coli* cells, Genes Dev. 26 (2012) 2659–2667.
[30] W.H. DePas, D.A. Hufnagel, J.S. Lee, L.P. Blanco, H.C. Bernstein, S.T. Fisher, G.A. James, P.S. Stewart, M.R. Chapman, Iron induces bimodal population development by *Escherichia coli*, Proc. Natl. Acad. Sci. U. S. A. 110 (2013) 2629–2634.
[31] E.A. Epstein, M.A. Reizian, M.R. Chapman, Spatial clustering of the curlin secretion lipoprotein requires curli fiber assembly, J. Bacteriol. 191 (2009) 608–615.
[32] N. Grantcharova, V. Peters, C. Monteiro, K. Zakikhany, U. Romling, Bistable expression of CsgD in biofilm development of *Salmonella enterica* serovar typhimurium, J. Bacteriol. 192 (2010) 456–466.
[33] D.O. Serra, A.M. Richter, G. Klauck, F. Mika, R. Hengge, Microanatomy at cellular resolution and spatial order of physiological differentiation in a bacterial biofilm, MBio 4 (2013) e00103–e00113.
[34] U. Gerstel, U. Romling, Oxygen tension and nutrient starvation are major signals that regulate agfD promoter activity and expression of the multicellular morphotype in *Salmonella* typhimurium, Environ. Microbiol. 3 (2001) 638–648.
[35] A. Olsen, A. Arnqvist, M. Hammar, S. Normark, Environmental regulation of curli production in *Escherichia coli*, Infect. Agents Dis. 2 (1993) 272–274.
[36] C. Prigent-Combaret, O. Vidal, C. Dorel, P. Lejeune, Abiotic surface sensing and biofilm-dependent regulation of gene expression in *Escherichia coli*, J. Bacteriol. 181 (1999) 5993–6002.
[37] A.P. White, D.L. Gibson, W. Kim, W.W. Kay, M.G. Surette, Thin aggregative fimbriae and cellulose enhance long-term survival and persistence of *Salmonella*, J. Bacteriol. 188 (2006) 3219–3227.
[38] S.K. Collinson, S.C. Clouthier, J.L. Doran, P.A. Banser, W.W. Kay, Characterization of the agfBA fimbrial operon encoding thin aggregative fimbriae of *Salmonella enteritidis*, Adv. Exp. Med. Biol. 412 (1997) 247–248.
[39] K.E. Rudd, EcoGene: a genome sequence database for *Escherichia coli* K-12, Nucleic Acids Res. 28 (2000) 60–64.
[40] A. Ishihama, Prokaryotic genome regulation: multifactor promoters, multitarget regulators and hierarchic networks, FEMS Microbiol. Rev. 34 (2010) 628–645.
[41] A. Arnqvist, A. Olsen, J. Pfeifer, D.G. Russell, S. Normark, The Crl protein activates cryptic genes for curli formation and fibronectin binding in *Escherichia coli* HB101, Mol. Microbiol. 6 (1992) 2443–2452.
[42] A. Bougdour, C. Lelong, J. Geiselmann, Crl, a low temperature-induced protein in *Escherichia coli* that binds directly to the stationary phase sigma subunit of RNA polymerase, J. Biol. Chem. 279 (2004) 19540–19550.
[43] P.K. Brown, C.M. Dozois, C.A. Nickerson, A. Zuppardo, J. Terlonge, R.r. Curtiss, MlrA, a novel regulator of curli (AgF) and extracellular matrix synthesis by *Escherichia coli* and *Salmonella enterica* serovar Typhimurium, Mol. Microbiol. 41 (2001) 349–363.
[44] H. Ogasawara, K. Yamamoto, A. Ishihama, Regulatory role of MlrA in transcription activation of csgD, the master regulator of biofilm formation in *Escherichia coli*, FEMS Microbiol. Lett. 312 (2010) 160–168.
[45] U. Romling, M. Rohde, A. Olsen, S. Normark, J. Reinkoster, AgfD, the checkpoint of multicellular and aggregative behaviour in *Salmonella* typhimurium regulates at least two independent pathways, Mol. Microbiol. 36 (2000) 10–23.
[46] E. Brombacher, C. Dorel, A.J. Zehnder, P. Landini, The curli biosynthesis regulator CsgD co-ordinates the expression of both positive and negative determinants for biofilm formation in *Escherichia coli*, Microbiology 149 (2003) 2847–2857.
[47] H. Ogasawara, K. Yamamoto, A. Ishihama, Role of the biofilm master regulator CsgD in cross-regulation between biofilm formation and flagellar synthesis, J. Bacteriol. 193 (2011) 2587–2597.
[48] K. Zakikhany, C.R. Harrington, M. Nimtz, J.C. Hinton, U. Romling, Unphosphorylated CsgD controls biofilm formation in *Salmonella enterica* serovar Typhimurium, Mol. Microbiol. 77 (2010) 771–786.
[49] S.M. Reshamwala, S.B. Noronha, Biofilm formation in *Escherichia coli* cra mutants is impaired due to down-regulation of curli biosynthesis, Arch. Microbiol. 193 (2011) 711–722.
[50] T. Shimada, Y. Katayama, S. Kawakita, H. Ogasawara, M. Nakano, K. Yamamoto, A. Ishihama, A novel regulator RcdA of the csgD gene encoding the master regulator of biofilm formation in *Escherichia coli*, Microbiologyopen 1 (2012) 381–394.
[51] D. Zheng, C. Constantinidou, J.L. Hobman, S.D. Minchin, Identification of the CRP regulon using *in vitro* and *in vivo* transcriptional profiling, Nucleic Acids Res. 32 (2004) 5874–5893.
[52] C. Dorel, O. Vidal, C. Prigent-Combaret, I. Vallet, P. Lejeune, Involvement of the Cpx signal transduction pathway of *E. coli* in biofilm formation, FEMS Microbiol. Lett. 178 (1999) 169–175.
[53] G. Jubelin, A. Vianney, C. Beloin, J.M. Ghigo, J.C. Lazzaroni, P. Lejeune, C. Dorel, CpxR/OmpR interplay regulates curli gene expression in response to osmolarity in *Escherichia coli*, J. Bacteriol. 187 (2005) 2038–2049.
[54] L. Ferrieres, D.J. Clarke, The RcsC sensor kinase is required for normal biofilm formation in *Escherichia coli* K-12 and controls the expression of a regulon in response to growth on a solid surface, Mol. Microbiol. 50 (2003) 1665–1682.
[55] U. Romling, Z. Bian, M. Hammar, W.D. Sierralta, S. Normark, Curli fibers are highly conserved between *Salmonella* typhimurium and *Escherichia coli* with respect to operon structure and regulation, J. Bacteriol. 180 (1998) 722–731.
[56] C. Prigent-Combaret, E. Brombacher, O. Vidal, A. Ambert, P. Lejeune, P. Landini, C. Dorel, Complex regulatory network controls initial adhesion and biofilm formation in *Escherichia coli via* regulation of the csgD gene, J. Bacteriol. 183 (2001) 7213–7223.
[57] U. Gerstel, A. Kolb, U. Romling, Regulatory components at the csgD promoter—additional roles for OmpR and integration host factor and role of the 5′ untranslated region, FEMS Microbiol. Lett. 261 (2006) 109–117.
[58] U. Gerstel, C. Park, U. Romling, Complex regulation of csgD promoter activity by global regulatory proteins, Mol. Microbiol. 49 (2003) 639–654.
[59] H. Ogasawara, K. Yamada, A. Kori, K. Yamamoto, A. Ishihama, Regulation of the *Escherichia coli* csgD promoter: interplay between five transcription factors, Microbiology 156 (2010) 2470–2483.
[60] A. Olsen, A. Arnqvist, M. Hammar, S. Sukupolvi, S. Normark, 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 (1993) 523–536.
[61] E. Holmqvist, J. Reimegard, M. Sterk, N. Grantcharova, U. Romling, E.G. Wagner, Two antisense RNAs target the transcriptional regulator CsgD to inhibit curli synthesis, EMBO J. 29 (2010) 1840–1850.
[62] M.G. Jorgensen, M.K. Thomason, J. Havelund, P. Valentin-Hansen, G. Storz, Dual function of the McaS small RNA in controlling biofilm formation, Genes Dev. 27 (2012) 1132–1145.
[63] F. Mika, S. Busse, A. Possling, J. Berkholz, N. Tschowri, N. Sommerfeldt, M. Pruteanu, R. Hengge, Targeting of csgD by the small regulatory RNA RprA links stationary phase, biofilm formation and cell envelope stress in *Escherichia coli*, Mol. Microbiol. 84 (2012) 51–65.
[64] M.K. Thomason, F. Fontaine, N. De Lay, G. Storz, A small RNA that regulates motility and biofilm formation in response to changes in nutrient availability in *Escherichia coli*, Mol. Microbiol. 84 (2012) 17–35.
[65] C. Monteiro, K. Papenfort, K. Hentrich, I. Ahmad, S. Le Guyon, R. Reimann, N. Grantcharova, U. Romling, Hfq and Hfq-dependent small RNAs are major contributors to multicellular development in *Salmonella enterica* serovar Typhimurium, RNA Biol. 9 (2012) 489–502.
[66] F. Mika, R. Hengge, Small regulatory RNAs in the control of motility and biofilm formation in *E. coli* and *Salmonella*, Int. J. Mol. Sci. 14 (2013) 4560–4579.
[67] S.K. Collinson, S.C. Clouthier, J.L. Doran, P.A. Banser, W.W. Kay, *Salmonella enteritidis agfBAC* operon encoding thin, aggregative fimbriae, J. Bacteriol. 178 (1996) 662–667.
[68] D.L. Gibson, A.P. White, C.M. Rajotte, W.W. Kay, AgfC and AgfE facilitate extracellular thin aggregative fimbriae synthesis in *Salmonella enteritidis*, Microbiology 153 (2007) 1131–1140.
[69] M. Hammar, Z. Bian, S. Normark, Nucleator-dependent intercellular assembly of adhesive curli organelles in *Escherichia coli*, Proc. Natl. Acad. Sci. U. S. A. 93 (1996) 6562–6566.
[70] N.D. Hammer, J.C. Schmidt, M.R. Chapman, The curli nucleator protein, CsgB, contains an amyloidogenic domain that directs CsgA polymerization, Proc. Natl. Acad. Sci. U. S. A. 104 (2007) 12494–12499.
[71] M. Desvaux, M. Hebraud, R. Talon, I.R. Henderson, Secretion and subcellular localizations of bacterial proteins: a semantic awareness issue, Trends Microbiol. 17 (2009) 139–145.
[72] J.D. Taylor, Y. Zhou, P.S. Salgado, A. Patwardhan, M. McGuffie, T. Pape, G. Grabe, E. Ashman, S.C. Constable, P.J. Simpson, W.C. Lee, E. Cota, M.R. Chapman, S.J. Matthews, Atomic resolution insights into curli fiber biogenesis, Structure 19 (2011) 1307–1316.
[73] A.A. Nenninger, L.S. Robinson, N.D. Hammer, E.A. Epstein, M.P. Badtke, S.J. Hultgren, M.R. Chapman, CsgE is a curli secretion specificity factor that prevents amyloid fibre aggregation, Mol. Microbiol. 81 (2011) 486–499.
[74] A.A. Nenninger, L.S. Robinson, S.J. Hultgren, Localized and efficient curli nucleation requires the chaperone-like amyloid assembly protein CsgF, Proc. Natl. Acad. Sci. U. S. A. 106 (2009) 900–905.
[75] L.S. Robinson, E.M. Ashman, S.J. Hultgren, M.R. Chapman, Secretion of curli fibre subunits is mediated by the outer membrane-localized CsgG protein, Mol. Microbiol. 59 (2006) 870–881.
[76] S.K. Collinson, L. Emody, K.H. Muller, T.J. Trust, W.W. Kay, Purification and characterization of thin, aggregative fimbriae from *Salmonella enteritidis*, J. Bacteriol. 173 (1991) 4773–4781.
[77] I. Cherny, L. Rockah, O. Levy-Nissenbaum, U. Gophna, E.Z. Ron, E. Gazit, The formation of *Escherichia coli* curli amyloid fibrils is mediated by prion-like peptide repeats, J. Mol. Biol. 352 (2005) 245–252.
[78] X. Wang, M.R. Chapman, Sequence determinants of bacterial amyloid formation, J. Mol. Biol. 380 (2008) 570–580.

[79] R. Kayed, E. Head, J.L. Thompson, T.M. McIntire, S.C. Milton, C.W. Cotman, C.G. Glabe, Common structure of soluble amyloid oligomers implies common mechanism of pathogenesis, Science 300 (2003) 486–489.
[80] Y. Zhou, D. Smith, B.J. Leong, K. Brannstrom, F. Almqvist, M.R. Chapman, Promiscuous cross-seeding between bacterial amyloids promotes interspecies biofilms, J. Biol. Chem. 287 (2012) 35092–35103.
[81] X. Wang, Y. Zhou, J.J. Ren, N.D. Hammer, M.R. Chapman, Gatekeeper residues in the major curlin subunit modulate bacterial amyloid fiber biogenesis, Proc. Natl. Acad. Sci. U. S. A. 107 (2010) 163–168.
[82] X. Wang, N.D. Hammer, M.R. Chapman, The molecular basis of functional bacterial amyloid polymerization and nucleation, J. Biol. Chem. 283 (2008) 21530–21539.
[83] Z. Bian, S. Normark, Nucleator function of CsgB for the assembly of adhesive surface organelles in *Escherichia coli*, EMBO J. 16 (1997) 5827–5836.
[84] H. Loferer, M. Hammar, S. Normark, Availability of the fibre 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. 26 (1997) 11–23.
[85] A.P. White, S.K. Collinson, P.A. Banser, D.L. Gibson, M. Paetzel, N.C. Strynadka, W.W. Kay, Structure and characterization of AgfB from *Salmonella enteritidis* thin aggregative fimbriae, J. Mol. Biol. 311 (2001) 735–749.
[86] N.D. Hammer, B.A. McGuffie, Y. Zhou, M.P. Badtke, A.A. Reinke, K. Brannstrom, J.E. Gestwicki, A. Olofsson, F. Almqvist, M.R. Chapman, The C-terminal repeating units of CsgB direct bacterial functional amyloid nucleation, J. Mol. Biol. 422 (2012) 376–389.
[87] S.L. Bardy, J.R. Maddock, Polar explorations. Recent insights into the polarity of bacterial proteins, Curr. Opin. Microbiol. 10 (2007) 617–623.
[88] G. Ebersbach, C. Jacobs-Wagner, Exploration into the spatial and temporal mechanisms of bacterial polarity, Trends Microbiol. 15 (2007) 101–108.
[89] G.E. Soto, S.J. Hultgren, Bacterial adhesins: common themes and variations in architecture and assembly, J. Bacteriol. 181 (1999) 1059–1071.
[90] M.L. Evans, J.C. Schmidt, M. Ilbert, S.M. Doyle, S. Quan, J.C. Bardwell, U. Jakob, S. Wickner, M.R. Chapman, *E. coli* chaperones DnaK, Hsp33 and Spy inhibit bacterial functional amyloid assembly, Prion 5 (2011) 323–334.
[91] E.K. Andersson, C. Bengtsson, M.L. Evans, E. Chorell, M. Sellstedt, A.E.G. Lindgren, D.A. Hufnagel, M. Bhattacharya, P.M. Tessier, P. Wittung-Stafshede, F. Almqvist, M.R. Chapman, Modulation of curli assembly and pellicle biofilm formation by chemical and protein chaperones, Chem. Biol. (2013) (in press, PubMed ID: 24035282).
[92] G.G. Hardy, R.C. Allen, E. Toh, M. Long, P.J. Brown, J.L. Cole-Tobian, Y.V. Brun, A localized multimeric anchor attaches the *Caulobacter* holdfast to the cell pole, Mol. Microbiol. 76 (2010) 409–427.
[93] J.L. Cole, G.G. Hardy, D. Bodenmiller, E. Toh, A. Hinz, Y.V. Brun, The HfaB and HfaD adhesion proteins of *Caulobacter crescentus* are localized in the stalk, Mol. Microbiol. 49 (2003) 1671–1683.
[94] V. Sivanathan, A. Hochschild, A bacterial export system for generating extracellular amyloid aggregates, Nat. Protoc. 8 (2013) 1381–1390.
[95] B. O'Nuallain, R. Wetzel, Conformational Abs recognizing a generic amyloid fibril epitope, Proc. Natl. Acad. Sci. U. S. A. 99 (2002) 1485–1490.
[96] R. Kayed, E. Head, F. Sarsoza, T. Saing, C.W. Cotman, M. Necula, L. Margol, J. Wu, L. Breydo, J.L. Thompson, S. Rasool, T. Gurlo, P. Butler, C.G. Glabe, Fibril specific, conformation dependent antibodies recognize a generic epitope common to amyloid fibrils and fibrillar oligomers that is absent in prefibrillar oligomers, Mol. Neurodegener. 2 (2007) 18.
[97] A.R. Ladiwala, M. Bhattacharya, J.M. Perchiacca, P. Cao, D.P. Raleigh, A. Abedini, A.M. Schmidt, J. Varkey, R. Langen, P.M. Tessier, Rational design of potent domain antibody inhibitors of amyloid fibril assembly, Proc. Natl. Acad. Sci. U. S. A. 109 (2012) 19965–19970.
[98] J.M. Perchiacca, A.R. Ladiwala, M. Bhattacharya, P.M. Tessier, Structure-based design of conformation- and sequence-specific antibodies against amyloid beta, Proc. Natl. Acad. Sci. U. S. A. 109 (2012) 84–89.
[99] A. Svensson, A. Larsson, H. Emtenas, M. Hedenstrom, T. Fex, S.J. Hultgren, J.S. Pinkner, F. Almqvist, J. Kihlberg, Design and evaluation of pilicides: potential novel antibacterial agents directed against uropathogenic *Escherichia coli*, Chembiochem 2 (2001) 915–918.
[100] V. Aberg, F. Norman, E. Chorell, A. Westermark, A. Olofsson, A.E. Sauer-Eriksson, F. Almqvist, Microwave-assisted decarboxylation of bicyclic 2-pyridone scaffolds and identification of Abeta-peptide aggregation inhibitors, Org. Biomol. Chem. 3 (2005) 2817–2823.
[101] I. Horvath, C.F. Weise, E.K. Andersson, E. Chorell, M. Sellstedt, C. Bengtsson, A. Olofsson, S.J. Hultgren, M. Chapman, M. Wolf-Watz, F. Almqvist, P. Wittung-Stafshede, Mechanisms of protein oligomerization: inhibitor of functional amyloids templates alpha-synuclein fibrillation, J. Am. Chem. Soc. 134 (2012) 3439–3444.
[102] A. Arnqvist, A. Olsen, S. Normark, Sigma S-dependent growth-phase induction of the csgBA promoter in *Escherichia coli* can be achieved *in vivo* by sigma 70 in the absence of the nucleoid-associated protein H-NS, Mol. Microbiol. 13 (1994) 1021–1032.
[103] L.A. Pratt, T.J. Silhavy, Crl stimulates RpoS activity during stationary phase, Mol. Microbiol. 29 (1998) 1225–1236.
[104] O. Vidal, R. Longin, C. Prigent-Combaret, C. Dorel, M. Hooreman, P. Lejeune, Isolation of an *Escherichia coli* K-12 mutant strain able to form biofilms on inert surfaces: involvement of a new ompR allele that increases curli expression, J. Bacteriol. 180 (1998) 2442–2449.
[105] A. Vianney, G. Jubelin, S. Renault, C. Dorel, P. Lejeune, J.C. Lazzaroni, *Escherichia coli* tol and rcs genes participate in the complex network affecting curli synthesis, Microbiology 151 (2005) 2487–2497.
[106] H. Ogasawara, A. Hasegawa, E. Kanda, T. Miki, K. Yamamoto, A. Ishihama, Genomic SELEX search for target promoters under the control of the PhoQP-RstBA signal relay cascade, J. Bacteriol. 189 (2007) 4791–4799.
[107] X. Liu, P. De Wulf, Probing the ArcA-P modulon of *Escherichia coli* by whole genome transcriptional analysis and sequence recognition profiling, J. Biol. Chem. 279 (2004) 12588–12597.
[108] H. Ogasawara, S. Shinohara, K. Yamamoto, A. Ishihama, Novel regulation targets of the metal-response BasS–BasR two-component system of *Escherichia coli*, Microbiology 158 (2012) 1482–1492.