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Curli Biogenesis and Function

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Abstract

Curli are the major proteinaceous component of a complex extra-cellular matrix produced by many *Enterobacteriaceae*. Curli were first discovered in the late 1980s on *Escherichia coli* strains that caused bovine mastitis, and have since been implicated in many physiological and pathogenic processes of *E. coli* and *Salmonella* spp. Curli fibers are involved in adhesion to surfaces, cell aggregation, and biofilm formation. Curli also mediate host cell adhesion and invasion, and they are potent inducers of the host inflammatory response. The structure and biogenesis of curli are unique among bacterial fibers that have been described to date. Structurally and biochemically, curli belong to a growing class of fibers known as amyloids. Amyloid fiber formation is responsible for several human diseases including Alzheimer's, Huntington's, and prion diseases, although the process of in vivo amyloid formation is not well understood. Curli provide a unique system to study macromolecular assembly in bacteria and in vivo amyloid fiber formation. Here, we review curli biogenesis, regulation, role in biofilm formation, and role in pathogenesis.

Keywords

amyloid; cellulose; enteric; biofilm; extracellular matrix

INTRODUCTION

Bacteria are able to integrate and survive in a remarkably diverse collection of environments. In recent years, bacterial communities have been better appreciated as an integral part of most microbial lifestyles. These communities, or biofilms, are prominent during infections and are generally characterized by an extracellular matrix that can help sculpt three-dimensional structures, which promote the survival of its inhabitants in the face of environmental stresses (20,21). Community behavior is complex and can involve many genetic loci. Genetic loci involved in community behaviors often encode extracellular factors that promote surface colonization or cell-cell contact. Enteric bacteria such as *Escherichia coli* and *Salmonella* spp. express proteinaceous extracellular fibers called curli that are involved in surface and cell-cell contacts that promote community behavior and host colonization (3,31,32,88,89). Understanding the biogenesis of structures that promote biofilm formation, such as curli, is a prerequisite to the development of therapeutics that can attenuate biofilm formation and host colonization. Here, we discuss curli regulation and biogenesis and the role of these fibers in the lifestyle of enteric bacteria such as *E. coli*.

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CURLI BIOGENESIS

Curliated bacteria stain red when grown on plates supplemented with the diazo dye Congo red (CR), providing a convenient way to identify genes important for curli production (17). At least six proteins, encoded by the divergently transcribed *csgBA* and *csgDEFG* operons, are dedicated to curli formation in *E. coli* (33) (Figure 1). Homologous operons have been identified in *Salmonella* spp. and are called *agfBA* and *agfDEFG* (16,67). *Salmonella typhimurium agf* genes can complement mutations in *E. coli csg* genes (67). In *S. typhimurium* the fibers encoded by the *agf* operons are called Tafi (thin aggregative fimbrae). For the purposes of this review we refer to structures encoded by the *agf* and *csg* operons as curli.

The *csgBA* operon encodes the major structural subunit, CsgA, and the nucleator protein CsgB (33,34). A third gene, *csgC*, is in the *csgBA* operon, but no transcript for *csgC* has been detected and there is no reported role for CsgC in curli biogenesis (16,33). CsgA and CsgB are proteins of identical predicted size, share 30% sequence identity, and are built up of similar repeat motifs (34). In the absence of CsgB, curli are not assembled and the major subunit protein, CsgA, is secreted from the cell in an unpolymerized form (14,34). CsgA and CsgB do not have to be expressed from the same cell for curli assembly to occur. During a process called interbacterial complementation, a *csgB* mutant cell secretes soluble CsgA that can be assembled on the surface of a cell expressing only *csgB* (34) (Figure 2*a*). Interbacterial complementation is best illustrated with established nomenclature in which the strain that secretes soluble CsgA is the donor, and the strain that presents CsgB on its cell surface is the acceptor (Figure 2*a*). In *E. coli*, interbacterial complementation can work when strains are grown within a few millimeters of each other. However, in *Salmonella enterica* interbacterial complementation was not observed between donor and acceptor strains (86), suggesting an alternative mechanism for curli assembly. When mutations are made in lipopolysaccharide (LPS) O polysaccharide in *S. enterica,* interbacterial complementation can occur (87). The K-12 *E. coli* strains that were first used to study interbacterial complementation are LPS O polysaccharide deficient.

Interbacterial complementation has led to the hypothesis that curli assemble via the nucleation precipitation pathway (7,47). Nucleation precipitation is based on the idea that CsgA is secreted into the extracellular milieu and nucleated into a fiber by CsgB. However, until recently there was no evidence that CsgA is secreted from wild-type (Wt) cells in an unpolymerized form. Cherny and colleagues (65) showed that small, rationally designed peptides could abrogate curli formation when added to the media. These peptides are predicted to block reactive surfaces on CsgA that would prevent it from assembling into a fiber. Because the peptides are not thought to enter the cell, the assumption is that CsgA polymerization was blocked at the cell surface.

The *csgDEFG* operon encodes four accessory proteins required for curli assembly (33). CsgD is a positive transcriptional regulator of the *csgBA* operon and is discussed in more detail in the section on curli gene regulation. The roles of CsgE, CsgF, and CsgG are just beginning to be elucidated. CsgG is an outer membrane (OM) lipoprotein that is required for the stability and secretion of CsgA and CsgB (14,47). When streaked on CR-indicator plates, *csgG* mutants stain white and no fibers are visualized by electron microscopy (EM). *csgG* mutants do not act as acceptors or donors during interbacterial complementation, suggesting that no functional CsgA or CsgB is produced by these cells (64). CsgG interacts with itself and purified CsgG visualized by high-resolution EM forms oligomeric, ring-shaped complexes (Figure 3). These structures are analogous to those formed by other OM channel-forming proteins (10,77,78).

Overexpression of CsgG is also correlated with pore formation in the OM. Normally, gram-negative bacteria are resistant to erythromycin because this 741-Da antibiotic does not

efficiently cross the OM. However, if the integrity of the OM is breached, erythromycin can access the bacterial cytoplasm and poison translation. Expression of CsgG renders *E. coli* sensitive to erythromycin (64), suggesting that CsgG is able to permeabilize the OM. The possibility remains that CsgG only modifies the activity of another, yet unidentified, protein that is ultimately responsible for curli subunit translocation across the OM. Because CsgG has been purified to homogeneity, in vitro liposome swelling or voltage-gating experiments should determine whether CsgG could form pores in the absence of other proteins.

The CsgG-mediated secretion of CsgA is dependent on the N-terminal 22 amino acids of the mature CsgA protein. These residues are not predicted to be an integral part of the curli fiber (19). However, these residues are required for CsgA to be secreted and assembled into a fiber (64). When the N-terminal 22 amino acids of CsgA are fused to PhoA (alkaline phosphatase), the resulting CsgA/PhoA chimera forms a complex with CsgG at the OM. This suggests that the N-terminal 22 amino acids on CsgA provide specificity for secretion of the major curli subunit.

CsgE is a periplasmic protein and *csgE* mutants are defective in curli assembly as visualized by their inability to bind CR when grown on CR-indicator plates (14) (Figure 2*b*,*c*). CsgA and CsgB stability is greatly reduced in *csgE* mutants, which do not act as donors or acceptors during interbacterial complementation. *csgE* mutants produce a few CsgA fibers, but these fibers are morphologically distinct from those produced by Wt cells (14). CsgE also physically interacts with CsgG at the OM (64). *csgE* and *csgG* mutants have similar phenotypes and these two proteins likely work together to promote curli assembly, although the molecular role of CsgE is currently unknown.

CsgF is a periplasmic protein that also interacts with CsgG in the OM (64), but *csgF* mutants have a phenotype different from that of *csgE* mutants. *csgF* mutants stain pink when streaked onto CR-indicator plates (14) (Figure 2*b*,*c*). *csgF* mutants are phenotypically similar to *csgB* mutants because they secrete soluble, unpolymerized CsgA (14) and therefore act as donors, but not acceptors, during interbacterial complementation.

CURLI GENE REGULATION

The regulation of curli gene expression is extraordinarily complex and is responsive to many environmental cues (28). The intergenic region between the *csgDEFG* and the *csgBA* operons is one of the largest in *E. coli*. At the center of the curli regulatory network is the CsgD protein, which is a transcriptional regulator in the FixJ/UhpA family (33). CsgD positively regulates the *csgBA* operon (33), but unlike most transcriptional regulators it does not regulate its own expression (69). CsgD contains an N-terminal receiver domain and a C-terminal helix-turn-helix DNA binding domain. Although CsgD is proposed to regulate the *csgBA* promoter directly, there is no experimental evidence to demonstrate CsgD DNA binding activity. It is also unclear what stimulates CsgD expression and/or activity, but activation of CsgD is thought to result from phosphorylation of a conserved aspartic acid residue in the N-terminal receiver domain (69). Because CsgD is absolutely required for *csgBA* promoter activity, it is not surprising that regulators of CsgD expression influence *csgBA* expression.

One of the first conditions recognized to promote curli gene expression was growth at temperature below 30°C (2,54). For most laboratory strains of *E. coli* and most *Salmonella* strains, curli expression is best at temperatures below 30°C. However, it has been demonstrated that many clinical strains of *E. coli*, including sepsis isolates, can express curli at 37°C (6). Furthermore, mutations in the *csgD* promoter can result in strains that express curli regardless of temperature (70,81).

In addition to temperature, other environmental conditions also influence curli expression. Curli expression occurs maximally in media without salt (70). Nutrient limitation (nitrogen, phosphate, and iron) stimulates curli gene expression (27,70). Oxygen tension also plays a role in curli expression, with microaerophilic conditions resulting in maximal *csgD* transcription (27,67).

A number of regulatory systems contribute to the expression of the curli operons (Table 1). RpoS, the stationary-phase sigma factor, plays a key role in curli gene regulation both directly and indirectly (1,52). Curli genes are maximally expressed during stationary phase and their expression is dependent upon RpoS (1). Crl interacts with RpoS to facilitate RpoS binding to the *csgBA* promoter region, and therefore Crl is required in most strains for curli expression (2). However, some strains express curli independent of Crl (63). Crl and RpoS cooperatively regulate other stationary-phase-induced genes (60). Crl was proposed to be the thermal sensor that maximized curli operon expression at low temperatures (2), and it was recently shown that the Crl protein is more stable at lower temperatures (9). Selective stability of Crl at lower temperatures may explain the propensity of curli fibers to be maximally expressed below 30° C, although thermal regulation of curli expression occurs in strains that do no have the Crl protein (M. M. Barnhart & M. R. Chapman, unpublished observations). RpoS also modulates curli gene expression by activating MlrA expression, which is a positive transcriptional regulator of *csgD* (11).

Three two-component regulatory systems regulate curli gene expression: OmpR/EnvZ, CpxA/R, and Rcs (24,39,62,70,84,85). Of these two-component regulatory systems the OmpR/EnvZ has the most dramatic effect on curli gene regulation. The OmpR/EnvZ system responds to changes in osmolarity and regulates the porins OmpF and OmpC (59). EnvZ is the sensor kinase that senses a signal it transmits to the OmpR response regulator, which modulates gene expression. OmpR works by positively regulating *csgD* expression, and in an *ompR* mutant there is no *csgD* transcription (70). The *ompR234* allele, which has a leucine-to-arginine change at position 43, constitutively activates curli gene expression and promotes biofilm formation in strains that normally do not make biofilms (85).

The CpxA/R system is activated in response to envelope stress and/or misfolded periplasmic proteins, resulting in the upregulation of many periplasmic chaperones and proteases (36). CpxA is the sensor kinase and CpxR is the response regulator that modulates gene expression. CpxA/R negatively regulates both curli operons. Overexpression of *csgA* in the absence of a corresponding overexpression of *csgG* results in the activation of the Cpx pathway (23,61, 62). In fact, it has been difficult to experimentally detect the curli subunit proteins as they pass through the periplasm during curli assembly, suggesting that they are only transiently present in the periplasm. The Cpx pathway also regulates the P pilus system. P pilus subunits that misfold in the periplasm are recognized by the Cpx system and are rapidly degraded by the proteases present in the Cpx regulon (36,44,71). It is tempting to speculate that the Cpx system is at least partly responsible for the apparent instability of curli subunits in the periplasm.

Similar to Cpx, the Rcs pathway responds to membrane stress, specifically OM stress, and is best known for its positive regulatory effect on capsule synthesis (49,50). The Rcs pathway negatively regulates *csgD* expression (24,39,84). The Rcs pathway is also required for biofilm formation (24). Thus, regulation of the curli operons seems to be tightly coupled to the ability to form biofilms (23). It is interesting to note that both Rcs and Cpx negatively regulate curli operon expression. Therefore, curli may be important only during the initial stages of biofilm formation, possibly for initial adhesion, and then turned off as the Cpx and Rcs pathways become active during biofilm maturation.

Two global regulatory proteins, histone-like protein (HN-S) and IHF (integration host factor), have been implicated in curli gene expression. Both of these proteins modulate DNA architecture. In *S. typhimurium* deletion of *ihf* reduced *csgD* transcription and therefore curli production (28). The role of HN-S in curli gene expression is more complicated. In *S. typhimurium* deletion of *hns* results in a decrease in *csgD* transcription, suggesting that HN-S is a positive regulator of curli gene expression (26). However, in *E. coli* K-12 strains *hns* mutants cause an increase in *csgA* transcription, suggesting that HN-S negatively affects curli gene expression (1). These results underscore the complexity of curli gene expression.

CURLI ARE PART OF THE BACTERIAL EXTRACELLULAR MATRIX

Recently, it has been shown that many enteric bacteria express different morphotypes, which correspond to differences in the extracellular matrix that they produce (66,69,88). *S. typhimurium* and *E. coli* produce an extracellular matrix that features curli as the major proteinaceous component. Cellulose is a second component of the matrix, and a third polysaccharide component is proposed to be present, although its identity is unknown (87). Different morphotypes can be visualized by growing bacteria on CR-indicator plates. The four described morphotypes are rdar (red, dry, and rough; curli and cellulose), pdar (pink, dry, and rough; cellulose only), bdar (brown, dry, and rough; curli only), and saw (smooth and white; neither curli nor cellulose) (88,89) (Figure 4). MC4100, the K-12 *E. coli* strain that has been used to study curli assembly, does not produce cellulose, whereas pathogenic and commensal *E. coli* isolates can produce cellulose, curli, or both (8,88). CsgD plays an integral role in extracellular matrix production because it regulates curli gene expression and indirectly regulates cellulose production by activating *adrA* (33,69,89). AdrA synthesizes cyclic-di-GMP, which is required to produce cellulose (72), but does not regulate cellulose gene expression (89). Recently it has been demonstrated that cyclic-di-GMP is an important signaling molecule in bacteria, but exactly how it works to modulate gene expression has yet to be elucidated (22,68).

THE ROLE OF CURLI IN BIOFILM FORMATION

Biofilms, communities of bacteria that live together for the benefit of the group, are characterized by water channels, complex three-dimensional structures, and an increased resistance to environmental stresses. Curli are important for biofilm development in both *E. coli* and *Salmonella* spp. Biofilm formation is a multi-step developmental process that includes at least five distinguishable steps: (*a*) reversible attachment, (*b*) irreversible attachment and production of adhesive molecules such as exopolysaccharides and adhesions, (*c*) biofilm development characterized by a distinct mushroom shape, (*d*) biofilm maturation, and (*e*) biofilm dispersal. Many bacterial surface structures, including curli, flagella, pili, and exopolysaccharide, play roles in various aspects of biofilm development (83). Biofilms can be problematic in the food industry and hospital settings. Curli allow *Salmonella enteriditis* to adhere to Teflon and stainless steel, which can lead to biofilm formation and contamination of surfaces often used in the food industry (3).

In a screen used to identify genes that allow a nonadherent strain of *E. coli* to form biofilms, an allele of *ompR* called *ompR234* (L 43 R) that activated curli gene expression was identified (85). The ability of this strain to form a biofilm was dependent on *csgA*. These results suggested that curli were important in the initial stages of biofilm development during the attachment phase. Recent work has suggested that biofilms formed by curli-proficient strains have a morphology different from biofilms formed by curli-deficient strains. Curli-deficient strains form flat biofilms on polyurethane sheets, compared with the mature biofilms produced by curli-expressing strains (40). During biofilm formation, *E. coli* K-12 is able to produce curli

at 37°C, even though on agar plates or in static broth it is able to express curli at only 26°C (40).

CURLI ARE A BACTERIAL AMYLOID

The biochemical and structural properties of curli are fascinating. Curli share many distinguishing biochemical and structural properties with eukaryotic amyloid fibers. Amyloid fiber formation is traditionally associated with human diseases including Alzheimer's, Parkinson's, and prion diseases; however, curli are part of a growing number of functional amyloids (15). Like eukaryotic amyloid fibers, curli are nonbranching (14,54) (Figure 5), β-sheet rich fibers that are resistant to protease digestion and 1% sodium dodecyl sulfate (17-19). Regardless of their origin, all amyloid fibers cause a red shift when mixed with CR (42,43) and cause fluorescence when mixed with thioflavin T (ThT) (45,46). These two dyes have been used as a diagnostic for amyloid formation. Curli cause a red shift when mixed with CR and a 10- to 20-fold increase in ThT fluorescence (13,14). The hallmark of amyloid fibers is the conserved cross β-strand structure, in which condensed β-sheets are stacked parallel to the fiber axis and individual β-strands are perpendicular to the fiber axis (75,76). Computer modeling of the two curli subunits CsgA and CsgB predicts that they form a similar structure composed of five repeating strand-loop-strand motifs (19,86) (Figure 6). Each repeating unit is composed of conserved glycines, glutamines, and asparagines (Figure 6). Many eukaryotic amyloids are also rich in glutamine and asparagine residues (51,56). The glutamine and asparagine residues are predicted to form a hydrogen bond network that might contribute to the extreme stability of these fibers (57,58). Curli represent a novel twist to amyloid formation because mammalian amyloid formation is generally considered an off-pathway protein-folding event, but curli are the product of a highly regulated and directed process. In addition to curli, there is a growing list of functional amyloids that have been described in yeast, fungi, and mammals (7a,25a,76a,79a). Therefore, the amyloid fold is not just a biological mishap, but an important part of cellular physiology.

Westermark and colleagues (48) recently reported that injection of curli fibers into mice resulted in increased polymerization of the disease-associated amyloid protein A (some-times called secondary amyloid protein, or AA). AA fibril formation is a manifestation of chronic inflammatory disease, the result of which is severe tissue damage and morbidity. Like other amyloid-associated diseases, a key question in the pathogenesis of AA is, What are the underlying causes of the conversion of AA from a soluble protein to an amyloid fiber? The work of Westermark suggests that one possibility could be that heterologous amyloid fibers such as curli act as a seed to drive AA polymerization. *E. coli* and other enteric bacteria that express curli are found as part of our normal gut flora and many of these bacteria can also cause diseases such as sepsis, which might allow the direct interaction of AA and curli fibers.

ROLE OF CURLI IN PATHOGENESIS

Many extracellular surface fibers produced by bacteria are important in pathogenesis. A unifying role of curli in pathogenesis has not been elucidated, but several lines of evidence suggest that curli are important during the infectious process. Curli have been implicated in the attachment and invasion of host cells, interaction with host proteins, and activation of the immune system. Curli bind to the extracellular matrix proteins fibronectin (54) and laminin (52). However, the role of curli during host colonization may not be fully appreciated, because it was thought that curli were expressed only at temperatures below 30°C (54). It is now known that curli expression is strain and condition specific and that many enterics express curli under conditions found in the host. Curli are expressed in biofilms at 37°C (40) and mutations in the *csgD* promoter region can also result in curli expression at 37°C (70,81,82). Furthermore, many clinical isolates express curli at 37°C.

Curli bind to many host proteins (Table 2). Many of the proteins with which curli interact are proposed to facilitate bacterial dissemination through the host (6). Curli bind to the tissue-degrading enzyme plasminogen. Plasminogen is a serine protease that degrades fibrin and soft tissue and must be activated from its proenzyme form (12). Tissue type plasminogen activator (t-PA) activates plasminogen to plasmin. Curli bind to plasminogen and t-PA simultaneously, resulting in the activation of plasminogen to plasmin (73). By activating plasminogen, curliated bacteria might gain an advantage inside the host because this enzyme degrades soft tissue, which would allow the bacteria to gain access to deeper tissue.

Curliated bacteria and curli also bind to human contact-phase proteins including H-kininogen, fibrinogen, and factor XII (5,35,53). By binding to the contact-phase proteins, curliated bacteria slow clotting, which could increase the spread of bacteria to surrounding tissue (35). Curli have been implicated in sepsis because antibodies to CsgA were present in the sera from sepsis patients (6). Furthermore, bacteria isolated from these patients were capable of expressing curli at 37°C. Because curli bind to contact-phase proteins and plasminogen/t-PA, curliated bacteria may have an advantage in spreading throughout the body and thus might play a role in sepsis.

Curli also interact with molecules of the immune system. MHC class I, which present antigen to T cells, bind to curli (55). Curliated bacteria adhered better to tissue culture cells that overproduced MHC class I. However, curliated bacteria did not influence antigen processing and presentation (38). Recently, curli have been shown to be a pathogen-associated molecular pattern (PAMP) (80). PAMPs are molecules produced by pathogenic bacteria that are recognized by specific host proteins called Toll-like receptors (TLRs), resulting in the activation of the innate immune system (25). Pili, flagella, LPS, and peptidoglycan are PAMPs, each recognized by one or more TLR molecules, resulting in the activation of proinflammatory cytokines. Curli are recognized by TLR2, resulting in the activation of IL-8 (80). This is consistent with previous studies that demonstrated that curliated bacteria result in activation of IL-6, IL-8, and TNF-α (6).

ROLE OF CURLI IN ATTACHMENT AND INVASION OF HOST CELLS

Curli expressing *E. coli* and *Salmonella* spp. adhere to various eukaryotic cell lines better than noncurliated strains do. An *E. coli* K-12 strain expressing curli adheres better to uroepithelial cells than noncurliated strains do (40). Expression of the curli genes in a K-12 *E. coli* strain that normally does not express curli resulted in the invasion of human cervical epithelial (HeLa) cells (31). Invasion by K-12 isolates expressing curli can be inhibited by peptides that block curli formation (65). *E. coli* 0157:H7 strains that produce curli attach and invade human laryngeal epithelial (Hep-2) cells (41,82). Also CR binding variants of 0157:H7 strains have increased virulence in a mouse model (82). *S. typhimurium* SR-11-expressing curli mediate attachment to cultured mouse small intestinal epithelial cells (74). Taken together, these results suggest that curli play an important role in the initial stages of the infection process.

Interestingly, it has recently been demonstrated that curli can promote binding to plant cells (4,37,79). In some strains, curli are maximally expressed at 26°C, a temperature at which plants are grown. *E. coli* K-12 strains that overproduce curli adhere to alfalfa sprouts, but mutations in *csgA* or *csgD* do not prevent binding by *E. coli* 0157:H7 (37,79). This suggests that pathogenic isolates of *E. coli* have multiple ways to adhere to the plant cells, whereas K-12 isolates do not. In *S. enterica* a deletion of *csgB*, but not of *csgA*, decreased adherence to alfalfa sprouts (4). This suggested that CsgB might be important for bacterial adherence. Previous work demonstrated that when CsgB was overexpressed in a *csgA csgB* double mutant short fibers were visualized (7). However, when Wt levels of CsgB were expressed no fibers were detected. It is an interesting hypothesis that CsgB could be important for mediating attachment to plant cells. Because contaminated plants result in infections from *E. coli* 0157:H7 or *S.*

enterica, by understanding how these organisms interact with plants, measures might be taken to block attachment.

An interesting observation was made in which CsgA antisera was found in babies who died from sudden infant death syndrome (SIDS), but age-matched controls did not contain CsgA antiserum (29,30). No infectious agent has been demonstrated in SIDS, but it is interesting to speculate that curli or curliated bacteria could play some role.

CONCLUSIONS

Curli represent a fascinating system to study many aspects of biology. Curli are one of a growing number of naturally occurring amyloids. Amyloid fiber formation, once thought to be exclusively the result of off-pathway protein misfolding, is now appreciated as a frequently occurring and physiologically important protein fold. The sophistication of *E. coli* genetics should allow the curli biogenesis system to serve as an excellent model system for understanding the complex macromolecular interactions that promote or antagonize amyloid fiber formation. Curli also represent an excellent system for studying protein secretion and macromolecular assembly in gram-negative bacteria. We are just beginning to elucidate the functions of the nonstructural curli proteins. Future work will help determine the role of CsgE and CsgF in curli assembly and how these proteins interact with CsgG to modulate CsgA and CsgB function. Curli are tightly regulated and can also be used to understand the complex interplay among multiple regulatory pathways. Also, the role of curli in mediating biofilm formation is an ever-evolving field and it is sure to reveal new insights into the structures and developmental pathways required for community behavior.

SUMMARY POINTS

1. Assembled by enteric bacteria, curli are the proteinaceous component of an extracellular matrix that also includes cellulose.
2. Curli are a bacterial amyloid and therefore represent a unique model system to study amyloid fiber formation, along with bacterial protein secretion and macromolecular assembly.
3. The major curli subunit protein, CsgA, is nucleated into a fiber by the CsgB protein. The accessory proteins CsgE, CsgF, and CsgG facilitate the secretion and assembly of CsgA into a fiber.
4. The regulation of the *csg* operons is complex and responds to multiple environmental cues.
5. Curli are required during the initial stages of biofilm development, likely in the attachment phase.
6. Curli-proficient bacteria promote adherence to multiple cell lines. Curli fibers themselves interact with many host proteins and are potent inducers of the host inflammatory response.

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Glossary

CR	Congo red
csg	curli specific gene
LPS	lipopolysaccharide
Wt	wild type
OM	outer membrane
EM	electron microscopy
ThT	thioflavin T
AA	amyloid protein A
t-PA	tissue type plasminogen activator
PAMP	pathogen-associated molecular pattern
TLRs	Toll-like receptors

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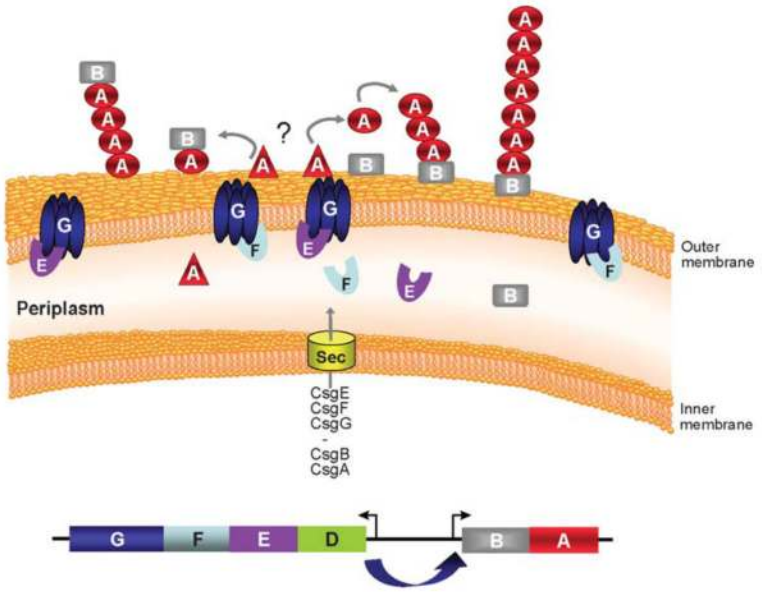

Figure 1. Model of curli assembly

A schematic diagram of the two curli gene operons is shown (*bottom*). CsgD is a positive transcriptional regulator of the *csgBA* operon. All the proteins encoded by the *csg* operons, except for CsgD, contain sec signal sequences for translocation into the periplasm. CsgG is an outer membrane protein required for the secretion of the two curli structural subunits CsgA and CsgB. CsgA is secreted outside of the cell where CsgB nucleates it into a fiber. CsgE and CsgF both interact with CsgG and are required for efficient curli assembly.

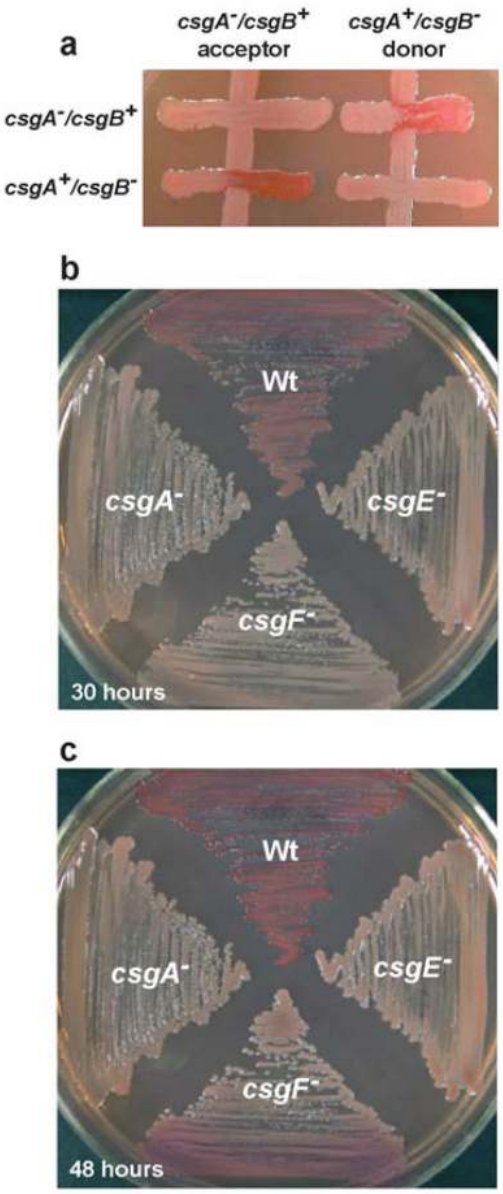

Figure 2. Phenotype of *csg* mutant strains after growth on CR-indicator plates

(*a*) A CR-indicator plate demonstrating interbacterial complementation. *csgA*$^{-}$/*csgB*$^{+}$ acceptor and *csgA*$^{+}$/*csgB*$^{-}$ donor strains were struck from top to bottom of a CR-indicator plate. *csgA*$^{-}$ and *csgB*$^{-}$ strains were struck from left to right on the same CR-indicator plate. When a *csgA*$^{-}$ strain is cross-streaked with a *csgB*$^{-}$ strain, CR binding occurs. A CR-indicator plate of wild-type (Wt), *csgA*$^{-}$, *csgE*$^{-}$, and *csgF*$^{-}$ strains is shown after (*b*) 30 and (*c*) 48 h of growth at 26°C.

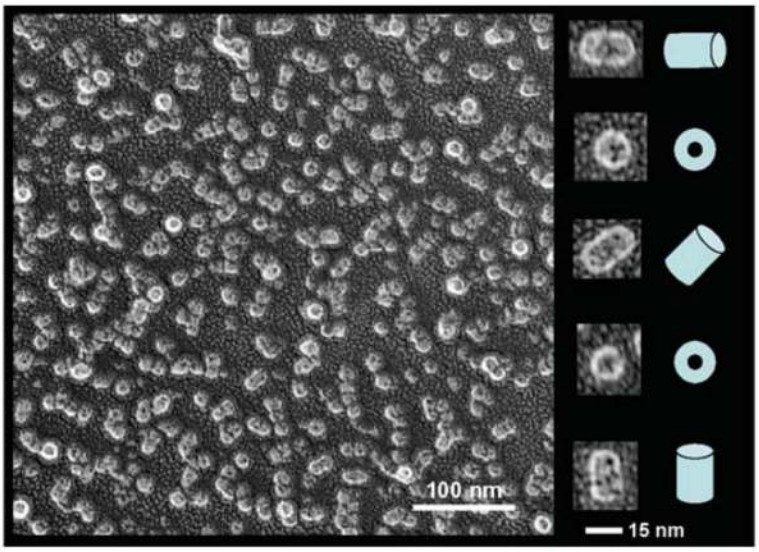

Figure 3. High-resolution EM of purified CsgG

Purified CsgG was visualized by high-resolution EM (*left*). At least two shapes of CsgG were observed: donut-like structures, which might indicate a top or bottom view of CsgG oligomers, and cylinders, which might represent a side view of the CsgG oligomeric complex (*right*).

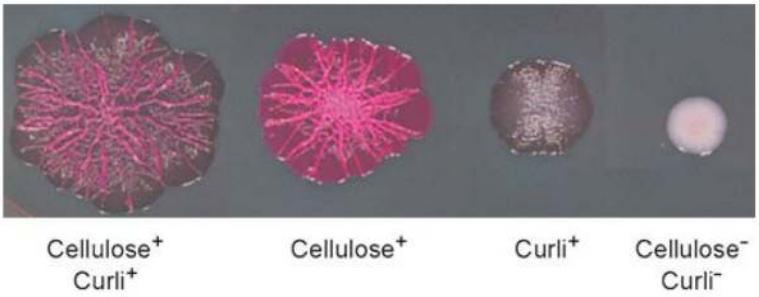

Figure 4.
Morphotypes of *Salmonella typhimurium* grown on CR-indicator plates for 48 h at 26°C. Figure was kindly provided by Ute Romling.

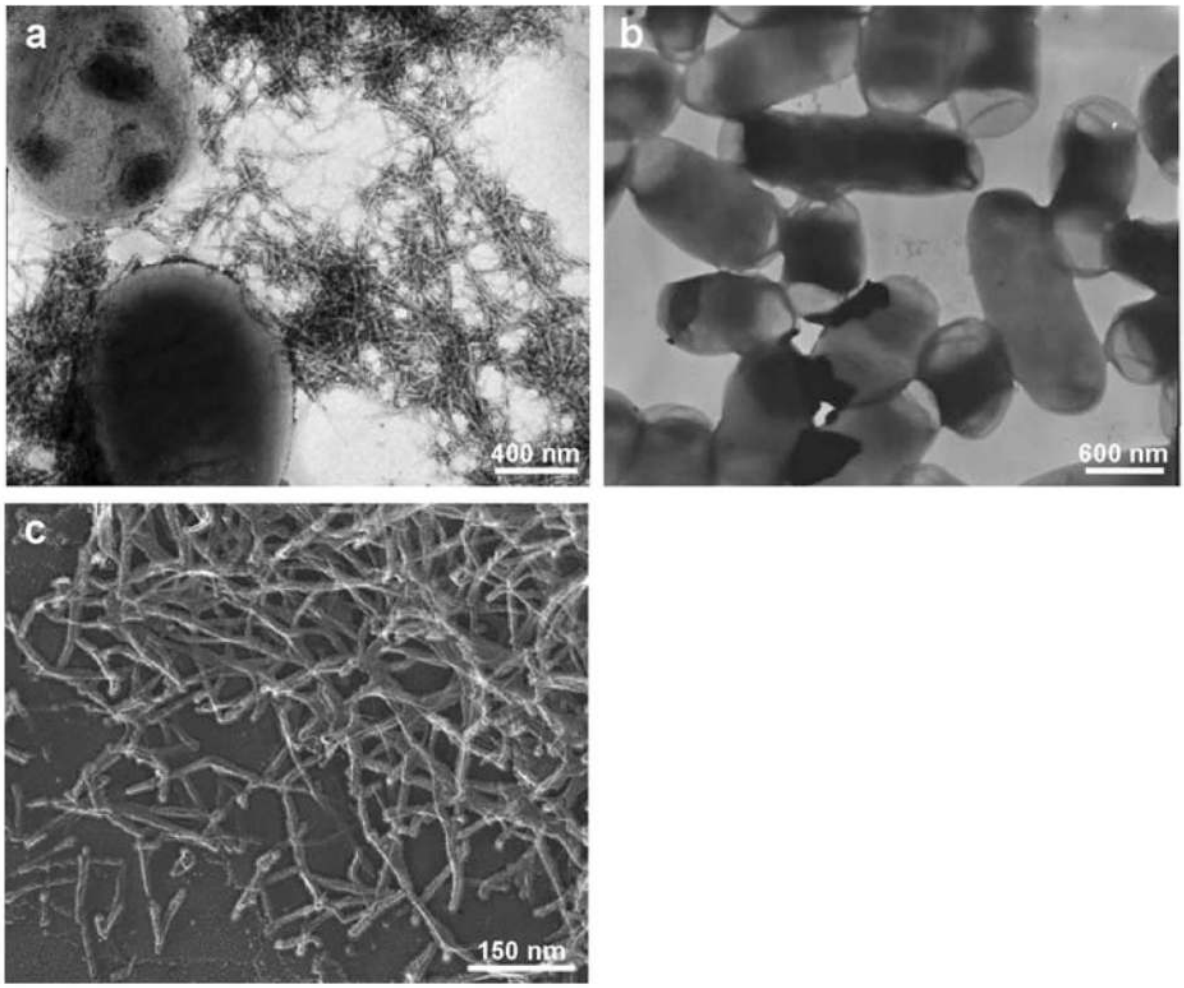

Figure 5. Electron micrographs of curli

Negative-stain EMs of (*a*)a bacterium-expressing curli and (*b*) bacteria not expressing curli are shown. (*c*) High-resolution EM of purified CsgA fibers.

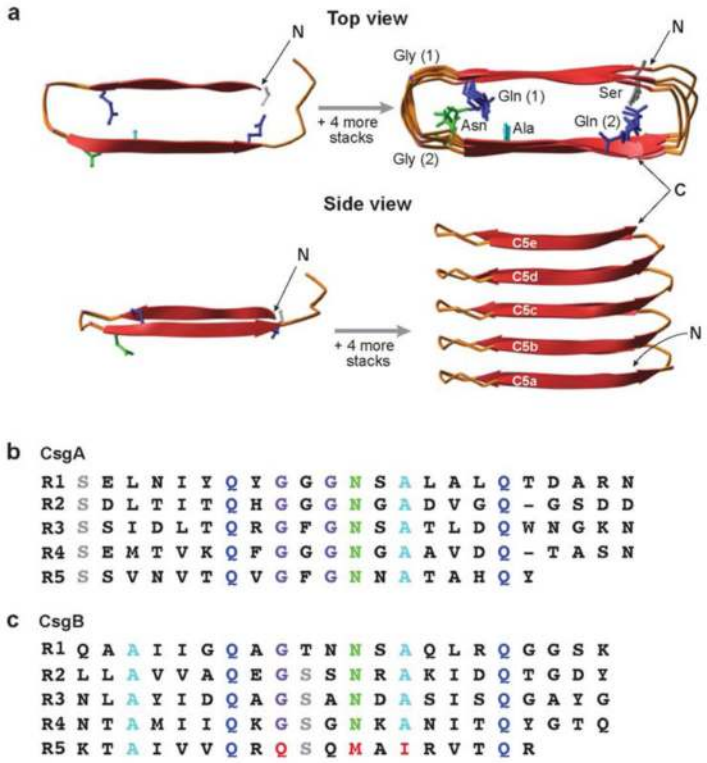

Figure 6. Model of CsgA and CsgB structure

(*a*)A model of the predicted strand-loop-strand motif of CsgA (19). A similar prediction was made for CsgB (86). (*b*) CsgA and (*c*) CsgB are composed of five repeating units (R1–R5), and each repeating unit is the equivalent of one strand-loop-strand. Residues that are conserved within the repeating units are colored the same. The red residues in R5 of (*c*) CsgB indicate residues that differ from R1–R4 of CsgB.

Table 1

Regulators of curli gene expression

Regulator	P*csgBA*[a]	P*csgDEFG*[a]	Reference(s)
CsgD	+		33
OmpR/EnvZ		+	62,67,85
RpoS		**+**	1,9,52,60
Crl		**+**	
CpxA/R	−	−	24,39
Rcs	−	−	24,39,84
MlrA		+	11
IHF		**+**	26
HN-S		+ or –[b]	1,26,52

[a] Activation or repression of the promoter is indicated by a + or −, respectively. No + or − indicates that no effect on the promoter has been demonstrated. A bold + or − indicates that direct binding to the promoter was observed.

[b] The role of HN-S in curli gene expression is dependent on the strain background. In *E. coli* K-12 HN-S has a negative role (1,52), whereas in *S. typhimurium* HN-S positively influences curli gene expression (26).

Table 2

Proteins that interact with curli

Protein	Function	Reference(s)
Fibronectin	Extracellular matrix protein	17,54
Laminin	Extracellular matrix protein	52
MHC class I	Antigen-presenting molecule	55
TLR2	Innate immune system activation	80
Plasminogen	Serine protease that degrades soft tissue when activated to plasmin	73
t-PA	Converts plasminogen to plasmin	
H-Kininogen	Contact-phase proteins	5,35,53
Fibrinogen		
Factor XII		