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Promiscuous Cross-seeding between Bacterial Amyloids Promotes Interspecies Biofilms*[S]

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Yizhou Zhou[‡], Daniel Smith[‡1], Bryan J. Leong[‡], Kristoffer Brännström[§], Fredrik Almqvist[¶‖], and Matthew R. Chapman[‡‖2]

From the [‡]Department of Molecular, Cellular and Developmental Biology, University of Michigan, Ann Arbor, Michigan 48109-1048 and the [§]Medical Biochemistry and Biophysics, [¶]Department of Chemistry, Chemical Biological Center, and [‖]Umeå Center for Microbial Research, Umeå University, 901 87 Umeå, Sweden

Background: Seeding of eukaryotic amyloids is highly specific.
Results: Curli subunits from different bacteria can cross-seed and such interspecies interactions restore surface attachment and biofilm formation.
Conclusion: Curli cross-seeding is relaxed, which promotes interspecies biofilms.
Significance: This is the first study on cross-seeding of bacterial amyloids and will help define the roles of bacterial amyloids in multispecies biofilms.

Amyloids are highly aggregated proteinaceous fibers historically associated with neurodegenerative conditions including Alzheimers, Parkinsons, and prion-based encephalopathies. Polymerization of amyloidogenic proteins into ordered fibers can be accelerated by preformed amyloid aggregates derived from the same protein in a process called seeding. Seeding of disease-associated amyloids and prions is highly specific and cross-seeding is usually limited or prevented. Here we describe the first study on the cross-seeding potential of bacterial functional amyloids. Curli are produced on the surface of many Gram-negative bacteria where they facilitate surface attachment and biofilm development. Curli fibers are composed of the major subunit CsgA and the nucleator CsgB, which templates CsgA into fibers. Our results showed that curli subunit homologs from *Escherichia coli*, *Salmonella typhimurium* LT2, and *Citrobacter koseri* were able to cross-seed *in vitro*. The polymerization of *Escherichia coli* CsgA was also accelerated by fibers derived from a distant homolog in *Shewanella oneidensis* that shares less than 30% identity in primary sequence. Cross-seeding of curli proteins was also observed in mixed colony biofilms with *E. coli* and *S. typhimurium*. CsgA was secreted from *E. coli csgB*− mutants assembled into fibers on adjacent *S. typhimurium* that presented CsgB on its surfaces. Similarly, CsgA was secreted by *S. typhimurium csgB*− mutants formed curli on CsgB-presenting *E. coli*. This interspecies curli assembly enhanced bacterial attachment to agar surfaces and supported pellicle biofilm formation. Collectively, this work suggests that the seeding specificity among curli homologs is relaxed and that heterogeneous curli fibers can facilitate multispecies biofilm development.

Amyloids are β-sheet-rich proteinaceous fibrils traditionally associated with protein misfolding and cytotoxicity (1–3). Amyloid formation is the hallmark of many neurodegenerative diseases such as Alzheimer, Parkinson, and prion-based diseases. Recently a rapidly growing class of "functional amyloids" suggests that the amyloid-fold can be utilized to facilitate non-degenerative physiological tasks (4–6). A number of functional amyloids have been described, such as curli produced by *Escherichia coli* (7), TasA by *Bacillus subtilis* (8), and Pmel17 by mammalian cells (9). Despite having little similarity in primary structure, amyloids share biochemical and structural propensities. Amyloid fibers are characterized by cross-β-sheet structures, with each β-strand perpendicular to the fiber axis (1, 10–12). These fibers are extraordinarily stable, resistant to most denaturation treatments and protease K digestion (7, 13), and possess the distinct tinctorial ability of binding the dyes Congo red and thioflavin T (ThT)[3] (7, 14). Another common feature of amyloids is the nucleation-dependent kinetics of assembly, in which amyloid proteins polymerize into fibers after a lag phase followed by an exponential growth (15–17). Formation of an oligomeric nucleus or seeds is rate-limiting and is associated with amyloid toxicity (18, 19). The self-polymerization of amyloid proteins can be accelerated by the presence of preformed fibers or nucleators in a process called seeding (20, 21).

Most amyloidogenic proteins can be seeded by fibers derived from the same protein. In rare cases, one amyloidogenic protein can be cross-seeded by different amyloid fibers. Cross-seeding is considered a possible mechanism for diverse pathologies of amyloid diseases and prion infections (22–25). Cross-seeding was observed between the Alzheimer-associated peptide Aβ and islet amyloid polypeptide (25), as well as between Aβ and human prion element PrP (24). Additionally, $A\beta_{1-42}$ fibers have been reported to induce the formation of tau-containing filaments *in vivo* (26), and *in vitro* preformed $A\beta_{1-42}$ oligomers

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[1] Present address: Division of Infectious Diseases, Children's Hospital Boston, 300 Longwood Ave., Boston, MA 02115.
[2] To whom correspondence should be addressed: Dept. of Molecular, Cellular, and Developmental Biology, University of Michigan LSA, 830 North University, Ann Arbor, MI 48109. Tel.: 734-764-7592; Fax: 734-647-0884; E-mail: chapmanm@umich.edu.

[3] The abbreviations used are: ThT, thioflavin T; Aβ, amyloid β; TEM, transmission electron microscopy; HFIP, 1,1,1,3,3,3-hexafluoro-2-propanol.

can induce the conversion of tau monomers to β-sheet-rich, toxic oligomers (27). Also, functional amyloids curli and Sup35 can promote amyloid protein A amyloidosis, suggesting that interactions between heterogeneous amyloid proteins may be a risk factor for accelerating the onset of amyloid diseases (23). Cross-seeding is also observed between some of the mammalian and yeast prion species, providing a mechanism for prion transmission and prion-based disease infection (28, 29).

Although limited cross-seeding among diverse amyloids has been reported, these interactions typically occur with reduced efficiency or are often completely prevented by species barriers. Seeding and cross-species transmission of most mammalian prion and yeast prion proteins are highly specific (29–31). Strict species barriers are present among conserved yeast prion domains including closely related Sup35 homologs from the *Saccharomyces sensu stricto* group (32, 33). A single amino acid mutation can alter the seeding specificity of Sup35 (34). Even the same Sup35 protein polymerizing at different temperatures forms fibers with distinct seeding specificity (35). Cross-seeding is also inefficient among mammalian prions (29), closely related synuclein homologs (36), different immunoglobulin domains (37), and lysozymes from different species (38).

Functional amyloids have been widely described in bacteria including *E. coli*, *Salmonella* spp., *B. subtilis*, *Streptomyces coelicolor*, and *Pseudomonas fluorescens* (7, 8, 39–41). Although cross-seeding among amyloid proteins has been extensively studied in disease-associated amyloids and prions, the seeding specificity of bacterial amyloids has not been investigated. To assess cross-seeding among functional bacterial amyloids, as well as the resultant biological consequences, we utilized the well studied bacterial functional amyloid called curli. Curli are amyloid fibers produced on the cell surface of *E. coli* and other enteric bacteria that facilitate adherence to biotic and abiotic surfaces (42, 43), biofilm development (39, 43–45), and pathogen-host interactions (46–48). Unlike disease-associated amyloids, curli assembly is highly regulated by dedicated pathways (49, 50). At least seven proteins, encoded by the *csgBAC* and *csgDEFG* operons (curli specific gene), are involved in curli biogenesis (4, 49). The major subunit of curli is CsgA. *In vivo*, the polymerization and membrane-localization of CsgA is dependent on the nucleator protein CsgB (51). The secretion of both CsgA and CsgB requires the outer-membrane pore-forming protein CsgG (52) and chaperone proteins CsgE and CsgF (53, 54). *In vitro* CsgA and CsgB self-assemble into amyloid fibers (51, 55) with β-helix structures (12). The *in vitro* fibrillization of CsgA can be seeded by its own fibers or by fibers of the nucleator protein CsgB (51, 55).

We report here that CsgA from *E. coli*, *Salmonella enterica* serovar *Typhimurium* LT2, *Citrobacter koseri*, and even a distant CsgA homolog from *Shewanella oneidensis* MR-1 are able to cross-seed *in vitro*. *In vivo*, both *S. typhimurium* and *E. coli* share curli subunits as building blocks to assemble functional fibers in colony biofilms and such interspecies interactions of curli subunits aid in bacterial adherence to abiotic surfaces and restore biofilm formation. Our results suggest that seeding between curli homologs is relaxed and that cross-seeding between different bacteria has an impact on multispecies communities.

EXPERIMENTAL PROCEDURES

Bacterial Growth—Bacteria were grown in LB at 37 °C with overnight shaking. To induce curli expression, bacteria were grown on YESCA agar (1 g/liter of yeast extract, 10 g/liter of casamino acids, and 20 g/liter of agar) or YESCA-CR (50 μg/ml of Congo red and 1 μg/ml of Coomassie Blue) at 26 °C for 48 h. To induce pellicle biofilm formation, bacteria were inoculated in 4 ml of static LB-no salt broth (10 g/liter of tryptone and 5 g/liter of yeast extract) at 26 °C for 3 days. Antibiotics were added at the following concentration: kanamycin, 50 μg/ml, and ampicillin, 100 μg/ml.

Strains and Plasmid—Strains and plasmids used in this study are listed in supplemental Tables S1 and S2. Primer sequences are listed in supplemental Table S3. *S. typhimurium* curli mutants were constructed according to the methods described by Datsenko and Wanner (56). All the *S. typhimurium* curli mutants can be complemented by expressing *S. typhimurium* CsgA or CsgB under the control of *S. typhimurium csgBA* promoter from the plasmid pACYC177.

Protein Purification—Purification of CsgA/CsgB homologs was adapted from Cegelski *et al.* (57) and Wang *et al.* (58). Briefly, expression of C-terminal His_6-tagged CsgA or CsgB homologs without the Sec signal sequence in NEB3016 was induced at A_{600} 0.9 by 0.5 mM isopropyl 1-thio-β-D-galactopyranoside at 37 °C for 1 h. Bacteria were lysed in 8 M guanidine hydrochloride in 50 mM potassium phosphate buffer (P_i) overnight. After centrifugation at 10,000 × *g* for 20 min the supernatant was incubated with nickel-nitrilotriacetic acid resin (Sigma) at room temperature for 1 h and then loaded onto a disposable polypropylene column (Thermo). Proteins were eluted into 50 mM potassium P_i containing 125 mM imidazole. To get monomeric CsgA, fractions with the target protein were combined and loaded onto a 30-kDa centrifugal filter units (Thermo) to remove dimers and other oligomers.

$A\beta_{1-42}$ Disaggregation—100% Trifluoroacetic acid (TFA) was added to $A\beta_{1-42}$ peptides to a 1 mg/ml ratio and bath sonicated at room temperature for 10 min. TFA was removed by a SpeedVac at room temperature for 1 h. Residual TFA was removed by dissolving the pellet in 500 μl of 1,1,1,3,3,3-hexafluoro-2-propanol (HFIP) and SpeedVac drying for 1 h; the process was repeated 3 times. The peptides were dissolved in 2 mM cold potassium hydroxide to 62.5 μM and cold 5× potassium P_i buffer was immediately added. Samples were centrifuged at 70,000 × *g* at 4 °C for 3 h and the top solution was taken carefully for the polymerization assay.

In Vitro Polymerization and Seeding Assay—100 μl of freshly purified CsgA homologs were loaded on a 96-well opaque plate and polymerization kinetics were monitored by ThT fluorescence with excitation wavelength at 438 nm and emission at 495 nm (bandwidth 20 nm) on a Tecan plate reader. ThT fluorescence was normalized as described (55). Seeds were prepared by sonication of preformed fibers with three 15-s bursts on ice. ThT was added to a final concentration of 20 μM.

Stability Assay—22 μg Fibers were spun down and resuspended in 50 mM potassium P_i or 50 (v/v), 70, 80, 90, or 100% HFIP for 5 min and immediately dried on a SpeedVac for 3 h.

The resulting pellets were boiled in 2× SDS sample buffer for 5 min before being loaded on 15% SDS-PAGE gels.

Biacore Binding Assay—A BIAcore3000 (GE Healthcare) was used to monitor real-time interactions between monomers and fibers. A CM5 sensor chip was activated with 35 μl of 1:1(v/v) mixture of 0.4 M 1-ethyl-3-(3-dimethylpropyl)-carbodiimide and 0.1 M *N*-hydroxysuccinimide at a flow rate of 5 μl/min. Mature CsgA or CsgB fibers were sonicated (Sonicator XL2020, Misonix) with three 15-s bursts at power 2 and 50-s pauses between. These seeds were then diluted into acetate buffer to final concentrations of 3.5 μM for CsgB fibers and 2.6 μM for CsgA fibers. 55 μl of sonicated fibers were injected at a flow rate of 5 μl/min to allow the immobilization of 2500–3500 resonance units. Blank flow cells on the same chip were used as negative controls. After ligand immobilization, excessive reactive groups were deactivated with 35 μl of 1 M ethanolamine-HCl, pH 8.5, and the sensor chip was primed with 50 μM potassium P_i, pH 7.4. 40 μl of 0.25 μM monomeric *E. coli* CsgA or *S. typhimurium* CsgA was injected over the sensor chip surface at a flow rate of 50 μl/min and the response was recorded in resonance units. Elution of a mock purification from NEB3013 harboring an empty vector pET11d and monomeric $A\beta_{1-42}$ with a concentration higher than 1 μM were used as negative controls.

Western Blot Analysis—Western blot analysis of the whole cell lysates and bacteria with the underlying agar (plug) were performed as described previously (45, 58). For whole cell lysate, bacteria grown on YESCA agar at 26 °C for 48 h were suspended in 50 mM potassium P_i, pH 7.2, normalized by optical density at 600 nm (A_{600}), and pelleted down. The pellets were pre-treated with or without 70 μl of HFIP immediately followed by SpeedVac centrifugation for 30 min at 45 °C. For the plug, overnight cultures of bacteria were normalized by A_{600} and 4 μl of culture were spotted on thin YESCA agar, incubated at 26 °C for 48 h. 8-Millimeter circular plugs including bacteria colonies and the underlying agar were collected, treated with or without 150 μl of HFIP, and dried immediately by a SpeedVac. Proteins in whole cell lysates or plugs were separated by electrophoresis and transferred onto a polyvinylidene difluoride membrane. CsgA was probed by antiserum raised against purified *E. coli* curli fibers (Proteintech, Chicago, IL).

Transmission Electron Microscopy—A Philips CM100 transmission electron microscope was used to visualize bacteria samples that were prepared as previously described (58).

Mixed Colony Biofilms—Interspecies curli assembly assay in mixed colony biofilms was adapted from Chapman *et al.* (7) and White *et al.* (59). Briefly, overnight cultures of *E. coli* and *S. typhimurium* curli mutants were normalized by A_{600} and mixed at 1:1 (v/v) ratio. 4 μl of each sample were spotted on YESCA agar and incubated at 26 °C for 3 days. Curli formation was analyzed by Western blot and transmission electron microscopy.

Measurement of Bacterial Adhesiveness—Overnight cultures of *E. coli* and *S. typhimurium* were normalized by A_{600} and were mixed at 1:1 (v/v) ratio. Bacterial cultures were spread on 3 ml of YESCA agar on 12-well plates and incubated at 26 °C for 3 days. To determine the bacterial adhesiveness, 1 ml of phosphate-buffered saline (PBS) was added into each well, and plates were rocked vigorously on an orbital titer plate shaker at speed 5 for 30 min at room temperature. Nonadherent bacteria in PBS were removed and the cell densities were determined by A_{600}. Adherent bacteria were suspended in another 1 ml of PBS buffer with an inoculation loop and the A_{600} was measured. The percentage of adherent bacteria was calculated. To determine the adhesiveness of *S. typhimurium*, two approaches were used. *S. typhimurium* WT or mutants were transformed with mCherry-expressing plasmid pAH9 and *E. coli* cells were transformed with YFP-expressing plasmid pAH16. Both mCherry and YFP were driven by *Staphylococcus aureus sarA* P1 promoter, which was constitutively on in *E. coli* (60). Nonadherent *S. typhimurium* and adherent *S. typhimurium* were determined by mCherry signal with the excitation wavelength at 600 ± 9 nm and the emission wavelength at 630 ± 20 nm. Alternatively, nonfluorescence labeled *E. coli* and *S. typhimurium* were used for adhesiveness as described above. Bacteria collected before and after washing with PBS were diluted and plated out on YESCA-CR plates. *E. coli* curli mutants formed white colonies, whereas *S. typhimurium* curli mutants formed pink colonies. Colony forming units of pink colonies before and after washing were counted and the percentage of adhesive *S. typhimurium* was calculated.

Pellicle Biofilm Assay—Overnight cultures of *S. typhimurium* WT, *csgA*− and *csgBA*− mutants were diluted by 1:1,000 (v/v) into 4 ml of liquid LB-no salt medium. Freshly purified *E. coli* CsgA, sonicated *E. coli* CsgA fibers, or mock purification from cells harboring an empty vector was added. Bacteria were incubated at 26 °C without shaking for 3 days. The liquid was removed carefully and pellicles were stained with 0.1% crystal violet for visualization.

RESULTS

Curli Subunits of Different Bacteria Cross-seed in Vitro—To investigate the seeding specificity of curli amyloid, closely related homologs of *E. coli* CsgA from *S. typhimurium* and *C. koseri* (~70% identity) and a distant homolog from *S. oneidensis* MR-1 (28% identity) were purified and tested for their ability to cross-seed *E. coli* CsgA polymerization *in vitro*. For simplicity, we refer to the CsgA homologs from different organisms as $CsgA_{EC}$ (CsgA from *E. coli*), $CsgA_{ST}$ (*S. enterica* serovar *Typhimiuium*), $CsgA_{CK}$ (*C. koseri*), and $CsgA_{SO}$ (*S. oneidensis*).

Freshly purified $CsgA_{EC}$ monomers spontaneously assemble into fibers with identifiable lag, exponential and stationary phases that can be followed by thioflavin T fluorescence in real time (55). Addition of preformed $CsgA_{EC}$ fiber seeds completely eliminated the lag phase (Fig. 1*A*), which was consistent with previous observations (55). In the presence of 5% (w/w) preformed $CsgA_{ST}$ or $CsgA_{CK}$ seeds, $CsgA_{EC}$ fibrillated with no lag phase, indicating fibers of $CsgA_{ST}$ or $CsgA_{CK}$ efficiently cross-seeded $CsgA_{EC}$ polymerization (Fig. 1*A*). Like $CsgA_{EC}$, monomeric $CsgA_{ST}$ assembled into fibers with an approximately 4 h lag phase. The polymerization was accelerated by 5% (w/w) of its own fibers, $CsgA_{EC}$ fibers, or $CsgA_{CK}$ fibers (Fig. 1*B*). The decrease in ThT fluorescence after the exponential phase was possibly due to the adhesion of fiber aggregates to the wall of wells, a phenomenon we often observe. $CsgA_{CK}$ also self-polymerized and could be cross-seeded by seeds of $CsgA_{EC}$

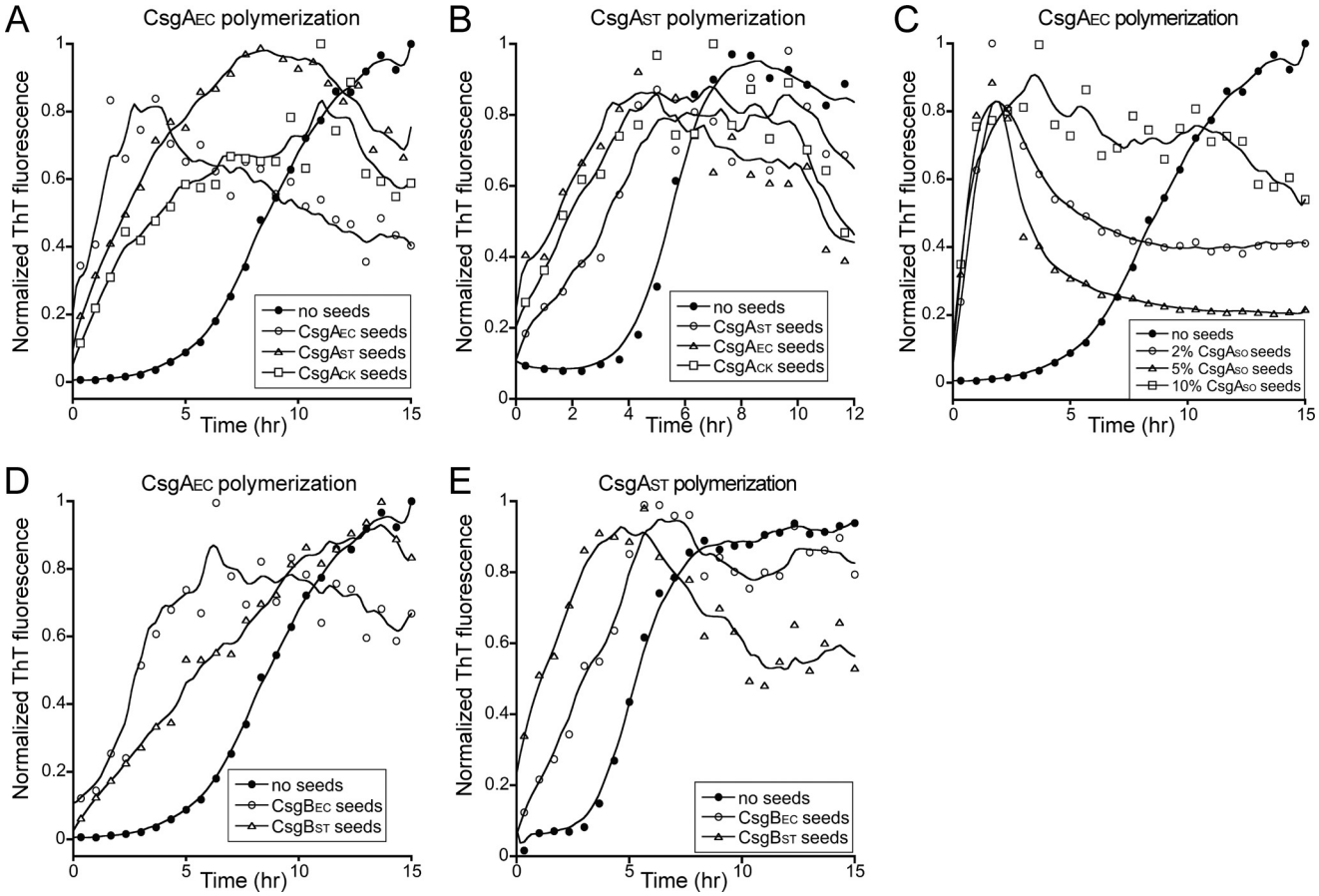

FIGURE 1. **Curli subunits cross-seeded *in vitro*.** *A,* normalized ThT fluorescence monitoring the polymerization kinetics of 10 μM *E. coli* CsgA ($CsgA_{EC}$) alone (●), or in the presence of 5% (w/w) sonicated $CsgA_{EC}$ seeds (○), *S. typhimurium* CsgA ($CsgA_{ST}$) seeds (Δ), or *C. koseri* CsgA ($CsgA_{CK}$) seeds (□). *B,* 10 μM freshly purified $CsgA_{ST}$ polymerized with no seeds (●), or in the presence of 5% sonicated $CsgA_{ST}$ seeds (○), $CsgA_{EC}$ seeds (Δ), or $CsgA_{CK}$ seeds (□). *C,* 10 μM $CsgA_{EC}$ polymerized alone (●), or in the presence of 2 (○), 5 (Δ), or 10% (□) *S. oneidensis* CsgA ($CsgA_{SO}$) seeds. *D,* 10 μM $CsgA_{EC}$ polymerized alone (●), with 5% $CsgB_{EC}$ seeds (○) or with 5% $CsgB_{ST}$ seeds (Δ). *E,* 10 μM $CsgA_{ST}$ polymerized alone (●), with 5% $CsgB_{EC}$ seeds (○) or with 5% $CsgB_{ST}$ seeds (Δ).

or $CsgA_{ST}$ (data not shown). Interestingly, the polymerization of $CsgA_{EC}$ was also seeded efficiently by a distant CsgA homolog from *S. oneidensis*. $CsgA_{SO}$ spontaneously assembled into amyloid-like fibers that were morphologically similar to *E. coli* curli fibers (supplemental Fig. S1*A*) and were rich in β-sheet secondary structure (supplemental Fig. S1*B*). The addition of preformed $CsgA_{SO}$ fibers effectively eliminated the lag phase of $CsgA_{EC}$ polymerization (Fig. 1*C*). Taken together, these results suggest that CsgA homologs from different bacteria efficiently cross-seed *in vitro*.

$CsgA_{EC}$ polymerization can also be seeded by *E. coli* CsgB ($CsgB_{EC}$) fibers *in vitro* (Fig. 1*D*). $CsgB_{EC}$ is proposed to quickly adopt an amyloid-fold and to template $CsgA_{EC}$ fiber formation *in vivo* (51). The amino acid sequences of $CsgA_{EC}$ and $CsgB_{EC}$ are less than 30% identical. Therefore, the interaction between CsgA and CsgB in *E. coli* represents a unique example of cross-seeding among amyloids (51, 61). It is unknown if CsgB has species-specific seeding determinants, or if CsgB can seed CsgA homologs from other bacterial species. To determine the seeding specificity of CsgB, $CsgB_E$ and CsgB homologs from *S. typhimurium* ($CsgB_{ST}$) were expressed and purified. 5% (w/w) $CsgB_{ST}$ fibers efficiently promoted the polymerization of $CsgA_{EC}$ (Fig. 1*D*). Similarly, 5% (w/w) $CsgB_{EC}$ seeds cross-seeded the polymerization of $CsgA_{ST}$ and $CsgA_{CK}$ (Fig. 1*E* and data not shown), suggesting that CsgB can cross-seed CsgA of a different bacteria.

We also measured the stability of fibers formed by CsgA homologs, CsgB homologs, or fibers formed by $CsgA_{EC}$ in the presence of various seeds in terms of HFIP resistance. HFIP is a strong denaturant that dissociates curli fibers into SDS-soluble monomers that migrate at 17 kDa on a SDS-PAGE gel (57). These fibers showed similar resistance to HFIP treatment: fibers were mostly resistant to 50, 70, or 80% HFIP treatment, and were largely dissociated with 90 or 100% HFIP treatment (supplemental Fig. S2). This result suggests that CsgA/CsgB fibers or those formed in the cross-seeding reaction may adopt similar conformations.

We further tested whether curli subunits can cross-seed with unrelated amyloidogenic peptides and proteins. The peptide amyloid $\beta(1-42)$ ($A\beta_{1-42}$) and the prion domain of yeast prion element Sup35 (Sup35 NM, which includes the N-terminal and the middle domain) were analyzed for their ability to cross-seed with *E. coli* CsgA. Neither $A\beta_{1-42}$ nor Sup35 NM was able to seed $CsgA_{EC}$, nor could the fibrillization of $A\beta_{1-42}$ or Sup35 NM be seeded by $CsgB_{EC}$ fibers (supplemental Fig. S3). The addition of $CsgA_{EC}$ seeds slightly increased the fibrillization of Sup35 NM, although to much lower levels than Sup35 NM seeded by its own fibers (supplemental Fig. S3). Therefore,

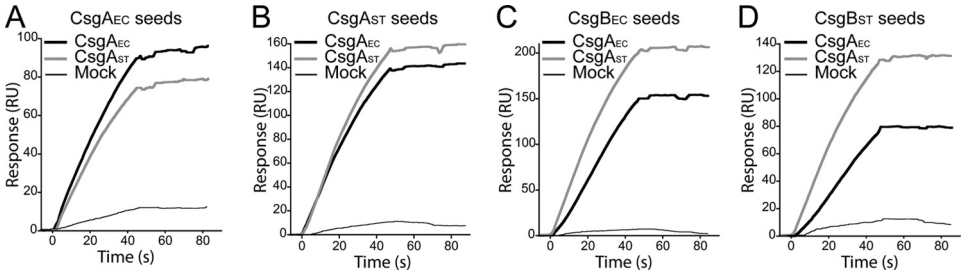

FIGURE 2. **Freshly purified CsgA bound preformed CsgA or CsgB seeds.** Surface plasmon resonance sensorgrams of 0.25 μM freshly purified $CsgA_{EC}$ (*black line*) and $CsgA_{ST}$ (*gray line*) or products from a mock CsgA purification from strains harboring an empty vector (*thin black line*) were injected over $CsgA_{EC}$ seeds (*A*), $CsgA_{ST}$ seeds (*B*), $CsgB_{EC}$ seeds (*C*), or $CsgB_{ST}$ (*D*) that were immobilized on a CM5 sensor chip.

although cross-seeding occurs between curli homologs, curli cannot cross-seed with unrelated amyloidogenic proteins.

CsgA Monomers Physically Bind Preformed Curli Fibers—To determine whether monomeric CsgA can physically interact with heterogeneous seeds or whether seed addition allows for adoption of a new fold without direct binding, surface plasmon resonance was performed to monitor interactions between CsgA monomers and preformed fibers in real time. Preformed $CsgA_{EC}$ fibers were immobilized on a sensor chip and 0.25 μM freshly purified $CsgA_{EC}$ or $CsgA_{ST}$ was injected over the chip. An increase in resonance units with no obvious signal decay was observed after the injection, suggesting a strong interaction between $CsgA_{EC}$ monomers and $CsgA_{EC}$ seeds, and between $CsgA_{ST}$ and $CsgA_{EC}$ seeds (Fig. 2*A*). The same response was detected when monomeric $CsgA_{EC}$ or $CsgA_{ST}$ was flowed over $CsgA_{ST}$ seeds, showing a strong association of polymeric $CsgA_{ST}$ with $CsgA_{ST}$ or $CsgA_{EC}$ monomers (Fig. 2*B*). Similarly, both freshly purified $CsgA_{EC}$ and $CsgA_{ST}$ bound to polymeric $CsgB_{EC}$ or $CsgB_{ST}$ (Fig. 2, *C* and *D*). As the control, BSA or mock purification from cells harboring an empty vector was flowed over polymeric $CsgA_{EC}$, $CsgA_{ST}$, $CsgB_{EC}$, or $CsgB_{ST}$ and no significant interaction was observed (Fig. 2 and data not shown). Finally, consistent with results shown in supplemental Fig. S3*B*, $A\beta_{1-42}$ only weakly interacted with $CsgA_{EC}$ seeds and quickly disassociated after injection (supplemental Fig. S4*A*). $A\beta_{1-42}$ did not interact with $CsgB_{EC}$ seeds at all (supplemental Fig. S4*B*). Together, these results suggest monomeric CsgA homologs directly bind fibers during cross-seeding reactions.

Mutations of Conserved Gln/Asn Residues Abolish the Cross-seeding of Curli—All the CsgA or CsgB homologs contain a C-terminal domain predicted to have imperfect β-strand-loop-β-strand repeating units and conserved Gln and Asn stacks (62). The first and last repeating units of *E. coli* CsgA are required for self-seeding and interactions between CsgA and CsgB (58, 63). Mutations of Gln and Asn residues in these repeating units to Ala result in a slow polymerizing variant of CsgA named $CsgA^{slowgo}$ ($CsgA^{Q49A,N54A,Q139A,N144A}$), which can be seeded by *E. coli* CsgA fibers, but cannot respond efficiently to CsgB-mediated heteronucleation (64), suggesting that CsgA self-seeding and heteronucleation may be mediated by distinct mechanisms. Interestingly, we found the conserved Gln and Asn residues were also necessary for cross-seeding between *E. coli* CsgA and other CsgA homologs. The polymerization of freshly purified $CsgA^{slowgo}$ was not efficiently seeded by fibers of $CsgA_{ST}$, $CsgA_{CK}$, or $CsgA_{SO}$ (Fig. 3 and data not shown). Consistent with previous results, 5% *E. coli* CsgA fibers completely eliminated the lag phase and promoted the polymerization of $CsgA^{slowgo}$ (Fig. 3). These results indicated that the conserved Gln and Asn residues in *E. coli* CsgA help mediate cross-seeding.

Interspecies Cross-seeding in Vivo—Like *E. coli*, *S. typhimurium*, *C. koseri*, and *S. oneidensis* all harbor the *csgDEFG* and *csgBA* operons required for curli biogenesis, and curli fibers were detected on the surface of *S. typhimurium* and *C. koseri* (65) (data not shown). The relaxed seeding of curli subunits *in vitro* led us to ask if cross-seeding of curli also occurred *in vivo* between different bacterial species.

To test if $CsgB_{EC}$ cross-seeds CsgA homologs under physiological conditions, we expressed $csgA_{ST}$, $csgA_{CK}$, and $csgA_{SO}$ driven by the *E. coli csgBA* promoter from a low copy plasmid in an *E. coli csgA*− mutant. As a control, we cloned the gene encoding $CsgA_{EC}$ into the same plasmid. If expression of CsgA homologs complements curli formation in an *E. coli csgA*− mutant, it would suggest that these homologs can be cross-seeded by $CsgB_{EC}$ on bacterial surfaces. Colonies formed by an *E. coli csgA*− mutant expressing $CsgA_{EC}$, $CsgA_{ST}$, or $CsgA_{CK}$ stained red on YESCA-CR plates, whereas the *csgA*− mutant with the vector control appeared white (Fig. 4*A*). Bacteria-associated $CsgA_{EC}$, $CsgA_{ST}$, and $CsgA_{CK}$ fibers were detected in whole cell lysates by Western blot after treatment with HFIP. $CsgA_{ST}$ and $CsgA_{CK}$ in the whole cell lysates were mostly SDS-insoluble, indicating that these CsgA homologs were incorporated into SDS-resistant curli fibers that could not migrate into SDS-PAGE (Fig. 4*C*). Curli-like fibers were observed on the *E. coli csgA*− mutant expressing $CsgA_{ST}$ and $CsgA_{CK}$ by electron microscopy (Fig. 4*D*). Thus, $CsgA_{ST}$ and $CsgA_{CK}$ complemented curli assembly in an *E. coli csgA*− mutant. Moreover, the complementation was CsgB dependent. *csgBA*−/p$CsgA_{ST}$ or *csgBA*−/p$CsgA_{CK}$ did not bind Congo red or produce fibers on the cell surface (Fig. 4, *B* and *E*), and no cell-associated $CsgA_{ST}$/$CsgA_{CK}$ was detected by Western blot. Instead, SDS-soluble $CsgA_{ST}$/Csg_{CK} was found in agar blocks underneath the colonies (Fig. 4*C*), indicating that CsgA homologs were secreted without polymerizing into fibers. Collectively, $CsgA_{ST}$ and $CsgA_{CK}$ can be seeded by $CsgB_{EC}$ on the cell surface. Additionally, an *E. coli csgB*− mutant could be complemented by expression of $CsgB_{ST}$ and $CsgB_{CK}$ (supplemental Fig. S5). Thus, $CsgA_{EC}$ could also be seeded by CsgB homologs from different bacteria. $CsgA_{SO}$ and $CsgB_{SO}$ were unable to complement *E. coli csgA*− or *E. coli csgB*− mutants (data not shown), possibly indicating that $CsgA_{SO}$ and $CsgB_{SO}$ are not properly expressed or localized in *E. coli*. Consistent with this we were

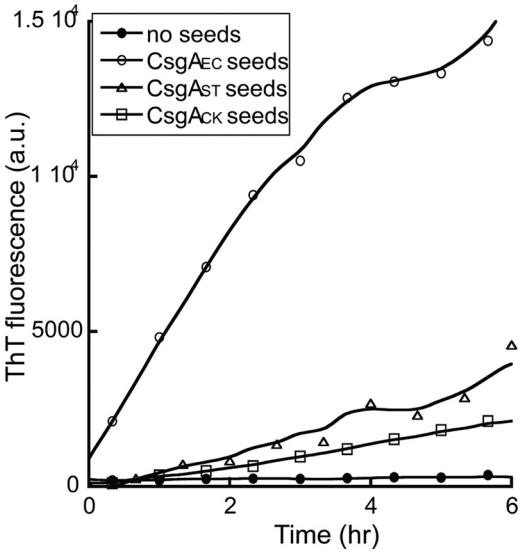

FIGURE 3. **Conserved Gln and Asn residues were required for cross-seeding between curli subunits.** ThT fluorescence of 30 μM freshly purified *E. coli* $CsgA^{slowgo}$ alone (●), or in the presence of 5% $CsgA_{EC}$ seeds (○), $CsgA_{ST}$ seeds (Δ), or $CsgA_{CK}$ seeds (□).

unable to detect expression of His-tagged $CsgA_{SO}$ in *E. coli* (data not shown).

We next asked if different bacteria grown in the same community could exchange curli subunits to assembled interbacterial curli fibers. Here we used *E. coli* and *S. typhimurium* because curli biogenesis is well understood in these two organisms and are proposed to frequently share the same ecological niche (65–67). During normal curli assembly in *E. coli*, CsgA is secreted unpolymerized into the extracellular environment prior to incorporation into fibers (7, 55). Soluble CsgA produced by an *E. coli csgB*− mutant (A^+B^-) can assemble into fibers on an adjacent *E. coli csgA*− mutant (A^-B^+) (7). In *S. enterica* serovar *Enteritidis*, such interbacterial curli assembly was detected only when the lipopolysaccharide O polysaccharide synthesis gene was mutated (59). To determine whether interspecies curli assembly occurred between *E. coli* and *S. typhimurium* (schematic in Fig. 5*A*), *E. coli* and *S. typhimurium csgA*−/*csgB*− mutants were mixed at a 1:1 (A_{600}/A_{600}) ratio and grown into mixed colonies. In a mixed colony with *E. coli csgB*− (A^+B^-) and *S. typhimurium csgA*− (A^-B^+), bacteria-associated, SDS-insoluble CsgA was readily detected by Western blot (Fig. 5*B*) and curli fibers were observed by TEM (Fig. 5*G*). Similar interbacterial curli assembly was detected in a mixed colony with *E. coli csgA*− (A^-B^+) and *S. typhimurium csgB*− (A^+B^-) by Western analysis (Fig. 5*B*) and TEM (Fig. 5*K*). It is interesting to notice that less curli were formed between *E. coli csgA*− (A^-B^+) and *S. typhimurium csgB*− (A^+B^-) than between *E. coli csgB*− (A^+B^-) and *S. typhimurium csgA*− (A^-B^+). This was probably because there were less *E. coli csgA*− (A^-B^+) cells to template $CsgA_{ST}$ into fibers. *S. typhimurium csgB*− (A^+B^-) outgrew *E. coli csgA*− (A^-B^+) by a ratio of 4:1 in mixed colonies, whereas *S. typhimurium csgA*− (A^-B^+) did not outcompete *E. coli csgB*− (A^+B^-) (supplemental Fig. S6*A*). Consistent with this notion, more SDS-soluble CsgA was detected in agar underneath the colony with *E. coli csgA*− (A^-B^+) and *S. typhimurium csgB*− (A^+B^-) (supplemental Fig. S6*B*), suggesting that some $CsgA_{ST}$ subunits were not incorporated into fibers and were secreted into the agar.

As controls, none of the single mutants produced curli on their own (Fig. 4*B*), and TEM (Fig. 5, *D*, *E*, *I*, and *J*) and curli were not detected in a mixture of *E. coli csgB*− (A^+B^-) and *S. typhimurium csgBA*− (A^-B^-) or *S. typhimurium csgB*− (A^+B^-) and *E. coli csgBA*− (A^-B^-), demonstrating that the nucleator CsgB was required to mediate interspecies curli assembly (date not shown). Consistent with previous reports (7, 59), interbacterial curli formed between *E. coli csgA*− and *E. coli csgB*− mutants (Fig. 5, *B* and *F*) but not between *S. typhimurium csgA*− and *csgB*− mutants (Fig. 5, *B* and *L*). However, curli formed by the two *E. coli* mutants was less resistant to SDS, suggesting either that those fibers may adopt a distinct conformation or that polymerization was less efficient. Together, both *E. coli* and *S. typhimurium* are capable of sharing curli subunits to build interspecies curli.

Interbacterial Complementation between E. coli Curli Mutants and S. typhimurium Curli Mutants Restore Bacterial Surface Attachment—The ability of *E. coli* and *S. typhimurium* to share curli subunits (Fig. 5) led us to further investigate the impact of such interspecies interactions on multispecies communities. Curli mediate surface attachment (42, 43) and bacteria-bacteria interactions (44). Wild-type *E. coli* formed a colony biofilm that tightly adhered to the agar surface even after vigorous shaking in PBS (Fig. 6*A*). The adhesiveness was dependent on curli production. Colonies of an *E. coli csgA*− mutant (A^-B^+) or an *E. coli csgB*− mutant (A^+B^-) did not strongly adhere to the agar surface; less than 40% of the curli-defective bacteria remained on agar after washing compared with 95% of WT (Fig. 6*A*). A *csgA*− mutant (A^-B^+) or a *csgB*− mutant (A^+B^-) of *S. typhimurium* also showed low adherence to the agar surface (Fig. 6*A*). Wild-type *S. typhimurium* has low surface adherence as well (Fig. 6, *A* and *B*), possibly due to the production of cellulose and other extracellular polysaccharides that counteract curli-mediated adherence (59, 68). However, over 80% of a mixed colony with *E. coli csgB*− (A^+B^-) and *S. typhimurium csgA*− (A^-B^+) stayed attached to the agar surface after vigorous washing with PBS (Fig. 6*A*). The increase in surface attachment was dependent on interspecies curli assembly, as mixed colonies with *E. coli csgB*− (A^+B^-) and *S. typhimurium csgBA*− (A^-B^-), or *S. typhimurium csgA*− (A^-B^+) and *E. coli csgBA*− (A^-B^-) mutants did not attach to the surface well (supplemental Fig. S7*A*). Consistent with the low level of interspecies curli production, we did not observe a significant increase in adherence when *E. coli csgA*− (A^-B^+) and *S. typhimurium csgB*− (A^+B-) was mixed (Fig. 6*A*). Collectively, these results demonstrate that curli assembled between *E. coli* and *S. typhimurium* are effective in restoring bacterial surface attachment.

Because *S. typhimurium* did not adhere to the agar surface as well as *E. coli*, we modified the assay to determine whether interspecies curli assembly between *E. coli* and *S. typhimurium* also facilitated surface attachment of *S. typhimurium*. To differentiate between the two species, a *S. typhimurium* strain carrying a plasmid that constitutively expresses mCherry was used. The adhesiveness of *S. typhimurium* was determined by mCherry fluorescence before and after the PBS wash. When

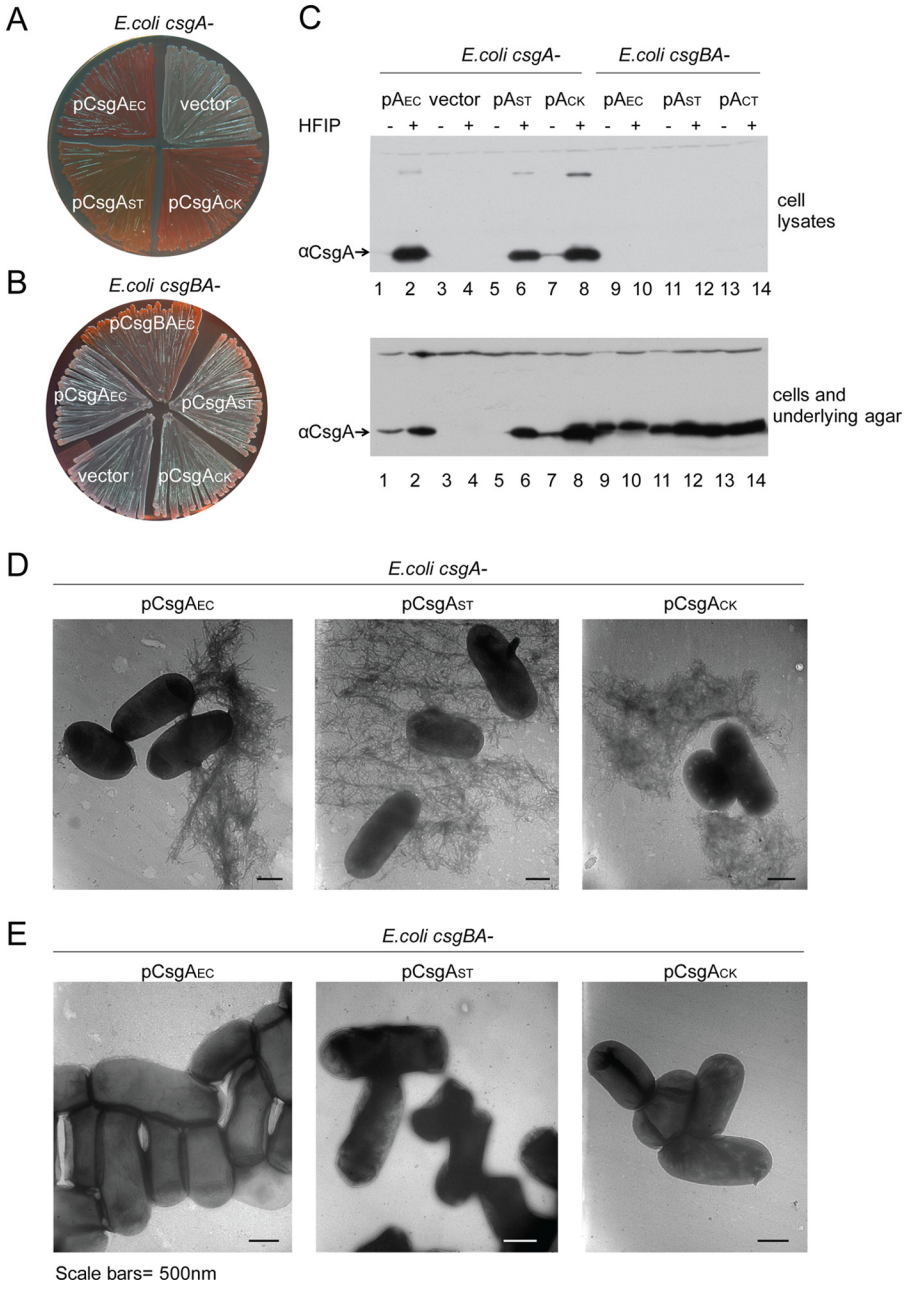

FIGURE 4. **An *E. coli csgA*− mutant was complemented by CsgA homologs from *S. typhimurium* or *C. koseri* in a CsgB-dependent manner.** *A,* the expression of CsgA homologs complemented the Congo red binding of an *E. coli csgA*− mutant. *E. coli csgA*− harboring an empty vector control or plasmids encoding $CsgA_{EC}$ ($pCsgA_{EC}$), $CsgA_{ST}$ ($pCsgA_{ST}$), or $CsgA_{CK}$ ($pCsgA_{CK}$) were grown on YESCA-CR plates at 26 °C for 48 h. *B*, CsgB was required for the complementation of Congo red binding. *E. coli csgBA*− mutant harboring an empty vector or plasmid $pCsgBA_{EC}$, $pCsgA_{EC}$, $pCsgA_{ST}$, or $pCsgA_{CK}$ were grown on a YESCA-CR agar at 26 °C for 48 h. *C,* Western blot of whole cell lysates (*top panel*) and plugs (*bottom panel*) of an *E. coli csgA*− mutant transformed with $pCsgA_{EC}$ (*lanes 1* and *2*), the vector control (*lanes 3* and *4*), $pCsgA_{ST}$ (*lanes 5* and *6*), or $pCsgA_{CK}$ (*lanes 7* and *8*), and an *E. coli csgBA*− mutant with $pCsgA_{EC}$ (*lanes 9* and *10*), $pCsgA_{ST}$ (*lanes 11* and *12*), or $pCsgA_{CK}$ (*lanes 13* and *14*) grown on YESCA agar plates at 26 °C for 48 h. Samples were treated with (+) or without (−) HFIP before electrophoresis and analyzed by aCsgA antibody. *D,* TEM of *E. coli csgA*− or *csgBA*− mutant transformed with $pCsgA_{EC}$, $pCsgA_{ST}$, or $pCsgA_{CK}$. *Scale bars* equal 500 nm.

mixed with *E. coli csgB*− (A^+B^-) mutant, the percentage of *S. typhimurium csgA*− (A^-B^+) mutant adhering to the agar surface increased by more than 60% (Fig. 6*B*). The increase in attachment was dependent on the nucleator CsgB; an *E. coli csgBA*− (A^-B^-) did not promote *S. typhimurium* adherence (Fig. 6*B*). These results were confirmed by the colony forming units of *S. typhimurium* in the mixed colony (supplemental Fig. S7*B*). Together, these results demonstrate that interspecies curli assembly between *E. coli* and *S. typhimurium* restores bacterial adhesiveness of the whole population and also promotes the attachment of *S. typhimurium* to agar.

Cross-seeding of Curli Restores Pellicle Biofilm Formation of S. typhimurium—Curli are important for the development of a pellicle biofilm, a type of biofilm that grows at the air-liquid interface (8, 45, 57). In static LB-no salt medium, *S. typhimurium* formed a pellicle biofilm (Fig. 7). The development of pellicle biofilms requires curli production (45), and indeed *S. typhimurium csgA*− or *csgBA*− mutants were unable to make

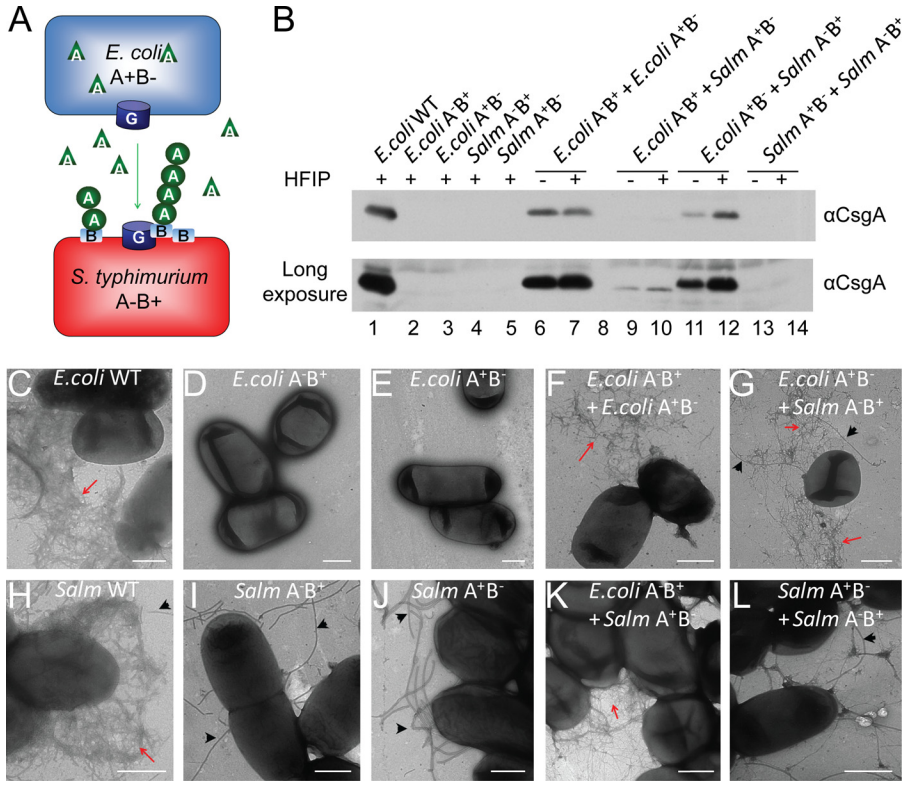

FIGURE 5. **Interbacterial curli assembly between *E. coli* and *S. typhimurium* curli mutants.** *A*, a schematic describing the interaction between an *E. coli csgB*− mutant (A^+B^-) and *S. typhimurium csgA*− mutant (A^-B^+). Unpolymerized *E. coli* CsgA secreted by *E. coli csgB*− (A^+B^-) is templated by CsgB on the surface of *S. typhimurium csgA*− (A^-B^+). *B*, Western blots of HFIP-treated (+) or nontreated (−) whole cell lysates of the indicated strains or strain mixtures. The blots were probed with aCsgA antibody. The *bottom panel* is a longer exposure of the same blot to visualize the faint bands. *C–L*, TEM of *E. coli* WT (*C*), *E. coli csgA*− (A^-B^+) (*D*), *E. coli csgB*− (A^+B^-) (*E*), a mixed colony with *E. coli csgA*− (A^-B^+) and *E. coli csgB*− (A^+B^-) (*F*), a mixture of *S. typhimurium csgA*− (A^-B^+) and *E. coli csgB*− (A^+B^-) (*G*), *S. typhimurium* WT (*H*), *S. typhimurium csgA*− (A^-B^+) (*I*), *S. typhimurium csgB*− (A^+B^-) (*J*), a mixture of *E. coli csgA*− (A^-B^+) and *S. typhimurium csgB*− (A^+B^-) (*K*), and a mixture of *S. typhimurium csgA*− (A^-B^+) and *S. typhimurium csgB*− (A^+B^-) (*L*). Curli fibers are indicated by *red arrows* and flagella are indicated by *black arrows*. *Scale bars* equal to 500 nm.

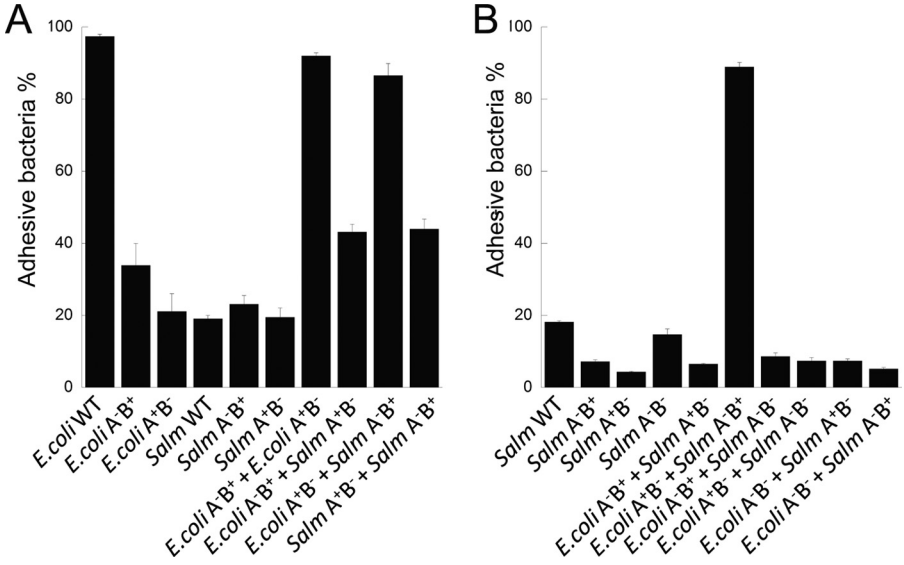

FIGURE 6. **Interbacterial curli formation between *E. coli* and *S. typhimurium* curli mutants restored bacteria adherence to agar surface.** *A*, overnight cultures of *E. coli*, *S. typhimurium*, or a 1:1 (A_{600}/A_{600}) mixture of *E. coli* and *S. typhimurium* curli mutants were spread on YESCA agar in 12-well tissue culture plates, then incubated at 26 °C for 3 days. Bacterial adhesiveness was determined by the percentage of adhered bacteria after vigorous washing as described under "Experimental Procedures." *B*, overnight cultures of *E. coli* constitutively expressing YFP from plasmid pAH16, *S. typhimurium* expressing constitutively expressing mCherry from pAH9 (60), or the mixed culture of *E. coli*/pAH16 and *S. typhimurium*/pAH9 were spread on YESCA agar on 12-well tissue culture plates, incubated at 26 °C for 3 days, and washed in PBS with vigorous shaking. Percentage of adhesive *S. typhimurium* was determined by mCherry signal with the excitation wavelength at 600 ± 9 nm and the emission wavelength at 630 ± 20 nm.

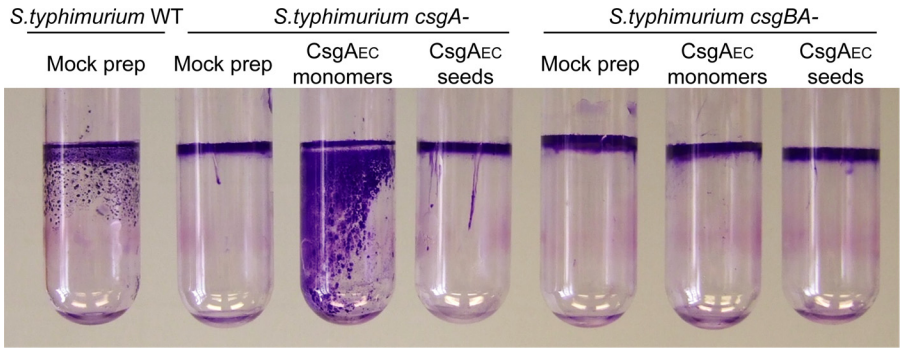

FIGURE 7. **Pellicle formation of *S. typhimurium csgA*− was complemented by exogenous *E. coli* CsgA subunits.** *S. typhimurium* WT, *csgA*−, or *csgBA*− mutants were incubated in static LB-no salt for 3 days at 26 °C in glass tubes. 10 μg/ml of freshly purified $CsgA_{EC}$ or $CsgA_{EC}$ seeds, or products from a mock CsgA purification from strains harboring an empty vector were added to each tube. The liquid culture underneath pellicles was removed and tubes were stained with crystal violet. When the liquid media was removed, pellicles fell against the wall of the glass tubes.

pellicles (Fig. 7). We asked if *S. typhimurium csgA*− could utilize and incorporate $CsgA_{EC}$ into fibers to support a pellicle biofilm. The addition of freshly purified $CsgA_{EC}$ monomers restored the pellicle development of the *S. typhimurium csgA*− mutant (Fig. 7). However, a *S. typhimurium csgBA*− mutant mixed with *E. coli* CsgA monomers did not form a pellicle (Fig. 7). Furthermore, sonicated, preformed *E. coli* CsgA seeds did not restore pellicle formation of the *S. typhimurium csgA*− mutant. This is probably because exogenously added fibers were not associated with cells and thus could not function as a scaffold to support pellicle biofilm formation. Thus, the cross-seeding of $CsgA_{EC}$ by $CsgB_{ST}$ on *S. typhimurium* cell surfaces facilitates pellicle biofilm development.

DISCUSSION

Amyloid proteins polymerize into fibers with nucleation-dependent kinetics. The conversion of monomers to an oligomeric nucleus contributes to the lag phase, but subsequently preformed fibers or nucleus can act as templates that accelerate amyloid formation. This process is known as seeding. Seeding is a critical step of amyloid propagation and disease development (1, 69). The work presented here demonstrates that bacterial functional amyloids have relatively relaxed seeding specificities. Amyloid cross-seeding of curli subunits was observed both *in vitro* between *E. coli*, *S. typhimurium*, *C. koseri*, and *S. oneidensis* as well as *in vivo* between *E. coli* and *S. typhimurium*. Remarkably this interspecies interaction facilitated bacterial surface attachment and biofilm development.

Amyloid seeding is typically highly specific. For instance, lack of cross-seeding is reported between the Parkinson disease-associated amyloid, α-synuclein, and closely related amyloidogenic homologs with more than 78% similarity (36). Species barriers are also commonly observed between different mammalian prion species, and between Sup35 homologs from different yeast species with up to 95% identity (29, 30, 32, 33). Even a single amino acid mutation could alter the seeding specificity of $A\beta_{1-42}$ and prions (25, 29). However, our results suggest that the cross-seeding specificity between curli subunits is relaxed. Efficient seeding was observed *in vitro* between curli subunits from a range of bacteria including *E. coli*, *S. typhimurium, C. koseri*, and *S. oneidensi*. Strikingly, although the $CsgA_{SO}$ is only 28% identical to *E. coli* CsgA in primary structure, it efficiently seeded $CsgA_{EC}$ amyloid formation with only 2% (w/w) seed concentration (Fig. 1*C*). We have also shown that during seeding and cross-seeding reactions, monomers physically interact with and bind fibers, as indicated by surface plasmon resonance (Fig. 2).

Curli assembly on bacterial surfaces is mediated by the nucleator protein CsgB. A longstanding model of curli biogenesis suggests that, once secreted, CsgB quickly adopts an amyloid fold that serves as a template to direct the assembly of CsgA into amyloid polymers (61, 70). The interaction between CsgA and CsgB in *E. coli* represents an example of heterogeneous seeding of amyloid proteins because *E. coli* CsgA and CsgB are less than 30% identical in amino acid sequence (51, 71). *In vitro*, both $CsgA_{EC}$ and $CsgB_{EC}$ spontaneously polymerize into amyloid fibers with a similar structure measured by CD and EM (51, 55). Shewmaker *et al.* (12) have also shown that both CsgA and CsgB adopt similar β-helix structure. Thus we hypothesize that CsgB-mediated templating is analogous to CsgA-mediated templating. CsgB-dependent heteronucleation could be mediated either by species-specific recognition sequences or by a promiscuous seeding mechanism. We have shown both *in vitro* and *in vivo* that $CsgB_{EC}$ can cross-seed $CsgA_{ST}$ and $CsgA_{CK}$, and in turn $CsgB_{ST}$ can cross-seed $CsgA_{EC}$ (Figs. 1, 2, and 4, and supplemental Fig. S5). Although cross-seeding between $CsgA_{EC}$ and other CsgB species was not tested due to the technical challenges in protein purification, curli formation by an *E. coli csgB*− mutant can be complemented by expressing $CsgB_{CK}$ in *trans* (supplemental Fig. S5), suggesting $CsgB_{CK}$ can also cross-seed the fibrillization of $CsgA_{EC}$. Therefore, CsgB-mediated seeding is likely to be a result of relaxed seeding of curli subunits.

Despite low sequence identity, both *E. coli* $CsgA_{EC}$ and $CsgB_{EC}$ are composed of an N-terminal Sec signaling sequence and a C-terminal domain with five 19–24 amino acid imperfect repeating units. Each repeating unit is predicted to adopt a β-strand-loop-β-strand motif and contains conserved Gln and Asn, which are critical for amyloid fiber formation (64). The same primary structure arrangement with regularly spaced Gln and Asn residues was also found in CsgA and CsgB homologs from other Gram-negative bacteria (62). Our previous results have shown that Gln and Asn residues in the N- and C-terminal repeating units (Gln-49, Asn-54, Gln-139, Asn-144) of *E. coli* CsgA are essential for efficient amyloid formation and interac-

tion with *E. coli* CsgB, as a $CsgA^{slowgo}$ mutant with those four Gln/Asn residues mutated cannot be cross-seeded by CsgB (64). In this study we further demonstrated that this mutant was also defective in cross-seeding with other CsgA homologs. Therefore these conserved Gln and Asn residues are important sequence determinants that mediate cross-seeding. An emerging consensus from studies of prion seeding specificity suggests that compatibility in protein or fiber conformations governs seeding specificity (35, 72–75). Not surprisingly, small changes in amino acid sequences can dictate conformational variability; therefore seeding specificity is tightly correlated with primary sequence (29, 64, 76). Specific side chain interactions also play an important role in amyloid formation and seeding (77). Gln and Asn residues in amyloid proteins form intra-molecular hydrogen bonds and help to stabilize the cross-β structure (77, 78). It is possible that the hydrogen bonds formed between Gln and Asn mediate interaction and seeding between different curli proteins. $CsgA^{slowgo}$ seeding with *E. coli* CsgA may be facilitated by other strain-specific side chain interactions.

The spatial arrangement of Gln and Asn within an amyloidogenic protein is proposed to play an important role in amyloid assembly (64). This could explain why the yeast prion Sup35, another Gln/Asn-rich amyloid protein, did not cross-seed with *E. coli* CsgA (supplemental Fig. S3), as the spacing between Gln and Asp in CsgA and in Sup35 are different. Moreover, circular dichroism spectra revealed that the secondary structures of CsgA fibers and Sup35-NM fibers are different, NM fibers have more random coil structure (7, 79) (data not shown), indicating that CsgA and Sup35 fibers may adopt incompatible conformations that limit the cross-seeding efficiency.

Although no cross-seeding was observed between curli and Sup35 or curli and $A\beta_{1-42}$ (supplemental Fig. S3), other eukaryotic amyloidogenic peptides including the human antimicrobial peptide LL-37 and amyloid protein A have been suggested to interact with curli directly or indirectly (23, 68). LL-37 is produced by epithelial cells of the urinary track to protect against infections with uropathogenic *E. coli* (80, 81). It is recently shown that LL-37 strongly bound to polymeric CsgA fibers (68), suggesting that curli may have the potential to promote the aggregation of LL-37 and bacteria might use cross-seeding as a strategy to counteract the antimicrobial effects. Amyloid protein A deposition is a result of chronic inflammations (82). Lundmark *et al.* (23) has shown that amyloid protein A deposition can be accelerated by amyloids including curli. Thus, cross-seeding may provide a mechanism for amyloidogenesis.

The highly specific seeding between disease-associated amyloids and prions is proposed to be a mechanism to prevent amyloid disease propagation and transmission. As a class of functional amyloids, the relaxed seeding propensity of curli may have an impact on biological events. Curli are an important component of enteric bacterial biofilm communities; mediating surface attachment and cell-cell interactions (43, 44, 57). Cross-seeding of curli subunits was observed *in vivo* in a mixed-species community, as interspecies curli assembly was found between *E. coli* and *S. typhimurium* curli mutants (Fig. 5). Moreover, interspecies assembled curli fibers were biologically functional. Intercellular curli assembly between *E. coli* and *S. typhimurium* curli mutants restored bacterial adherence to agar surfaces (Fig. 6).

Curli are also required for pellicle biofilm formation (57). A *S. typhimurium csgA*− mutant that cannot make a pellicle biofilm was able to utilize *E. coli* CsgA monomers to form a robust pellicle (Fig. 7). Romero *et al.* (8) recently showed that addition of *B. subtilis* amyloids TasA fibers restored the pellicle development of a *B. subtilis tasA* mutant. The addition of polymerized *E. coli* CsgA fibers did not restore pellicle formation by *S. typhimurium csgA*−, indicating that *S. typhimurium* cannot simply utilize curli fibers as a scaffold to support pellicle formation (Fig. 7). *E. coli* CsgA monomers or fibers were also unable to restore pellicle formation of a *S. typhimurium csgBA*− mutant that lacks the nucleator protein on its surface. Thus, the complementation of *S. typhimurium* pellicle formation by *E. coli* CsgA is dependent on the nucleation process.

In nature, bacterial communities are composed of multiple species (83, 84), and bacterial amyloids are abundant in natural biofilms (85). Because *E. coli* and *S. typhimurium* are proposed to share ecological niches, it is likely that these two species interact in biofilm environments (65, 67). Because curli subunits are secreted into the extracellular environment prior to assembly into polymers, and because, as an amyloid, curli can be cross-seeded, it is plausible that *E. coli* and *S. typhimurium* coexisting in natural communities can share curli subunits to build a heterogeneous matrix. Curli-like structures are also found in *Enterobacter* spp. *and Citrobacter* spp. (65, 79), and curli homologs are prevalent among *Pseudomon*as spp. and *Shewanella* spp. Thus the promiscuous seeding of curli may have a broad impact on the multispecies communities.

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REFERENCES

1. Chiti, F., and Dobson, C. M. (2006) Protein misfolding, functional amyloid, and human disease. *Annu. Rev. Biochem.* **75,** 333–366
2. Glenner, G. G., and Wong, C. W. (1984) Alzheimer disease. Initial report of the purification and characterization of a novel cerebrovascular amyloid protein. *Biochem. Biophys. Res. Commun.* **120,** 885–890
3. Prusiner, S. B. (1996) Molecular biology and pathogenesis of prion diseases. *Trends Biochem. Sci.* **21,** 482–487
4. Blanco, L. P., Evans, M. L., Smith, D. R., Badtke, M. P., and Chapman, M. R. (2012) Diversity, biogenesis and function of microbial amyloids. *Trends Microbiol.* **20,** 66–73
5. Badtke, M. P., Hammer, N. D., and Chapman, M. R. (2009) Functional amyloids signal their arrival. *Sci. Signal.* **2,** pe43
6. Fowler, D. M., Koulov, A. V., Balch, W. E., and Kelly, J. W. (2007) Functional amyloid. From bacteria to humans. *Trends Biochem. Sci.* **32,** 217–224
7. Chapman, M. R., Robinson, L. S., Pinkner, J. S., Roth, R., Heuser, J., Hammar, M., Normark, S., and Hultgren, S. J. (2002) Role of *Escherichia coli* curli operons in directing amyloid fiber formation. *Science* **295,** 851–855
8. Romero, D., Aguilar, C., Losick, R., and Kolter, R. (2010) Amyloid fibers provide structural integrity to *Bacillus subtilis* biofilms. *Proc. Natl. Acad. Sci. U.S.A.* **107,** 2230–2234
9. Fowler DM, K. A., Alory-Jost, C., Marks, M. S., Balch, W. E., Kelly, J. W. (2006) Functional amyloid formation within mammalian tissue. *PLoS Biol.*

4, 100–107
10. Sunde, M., and Blake, C. (1997) The structure of amyloid fibrils by electron microscopy and x-ray diffraction. *Adv. Protein Chem.* **50,** 123–159
11. Sunde, M., Serpell, L. C., Bartlam, M., Fraser, P. E., Pepys, M. B., and Blake, C. C. (1997) Common core structure of amyloid fibrils by synchrotron x-ray diffraction. *J. Mol. Biol.* **273,** 729–739
12. Shewmaker, F., McGlinchey, R. P., Thurber, K. R., McPhie, P., Dyda, F., Tycko, R., and Wickner, R. B. (2009) The functional curli amyloid is not based on in-register parallel β-sheet structure. *J. Biol. Chem.* **284,** 25065–25076
13. Nordstedt, C., Näslund, J., Tjernberg, L. O., Karlström, A. R., Thyberg, J., and Terenius, L. (1994) The Alzheimer A-β-peptide develops protease resistance in association with its polymerization into fibrils. *J. Biol. Chem.* **269,** 30773–30776
14. Ban, T., Hamada, D., Hasegawa, K., Naiki, H., and Goto, Y. (2003) Direct observation of amyloid fibril growth monitored by thioflavin T fluorescence. *J. Biol. Chem.* **278,** 16462–16465
15. Serio, T. R., Cashikar, A. G., Kowal, A. S., Sawicki, G. J., Moslehi, J. J., Serpell, L., Arnsdorf, M. F., and Lindquist, S. L. (2000) Nucleated conformational conversion and the replication of conformational information by a prion determinant. *Science* **289,** 1317–1321
16. Harper, J. D., Lieber, C. M., and Lansbury, P. T., Jr. (1997) Atomic force microscopic imaging of seeded fibril formation and fibril branching by the Alzheimer disease amyloid-β protein. *Chem. Biol*. **4,** 951–959
17. Walsh, D. M., Hartley, D. M., Kusumoto, Y., Fezoui, Y., Condron, M. M., Lomakin, A., Benedek, G. B., Selkoe, D. J., and Teplow, D. B. (1999) Amyloid β-protein fibrillogenesis. Structure and biological activity of protofibrillar intermediates. *J. Biol. Chem.* **274,** 25945–25952
18. Demuro, A., Mina, E., Kayed, R., Milton, S. C., Parker, I., and Glabe, C. G. (2005) Calcium dysregulation and membrane disruption as a ubiquitous neurotoxic mechanism of soluble amyloid oligomers. *J. Biol. Chem.* **280,** 17294–17300
19. Kayed, R., Sokolov, Y., Edmonds, B., McIntire, T. M., Milton, S. C., Hall, J. E., and Glabe, C. G. (2004) Permeabilization of lipid bilayers is a common conformation-dependent activity of soluble amyloid oligomers in protein misfolding diseases. *J. Biol. Chem.* **279,** 46363–46366
20. Glover, J. R., Kowal, A. S., Schirmer, E. C., Patino, M. M., Liu, J. J., and Lindquist, S. (1997) Self-seeded fibers formed by Sup35, the protein determinant of [PSI$^+$], a heritable prion-like factor of *S. cerevisiae*. *Cell* **89,** 811–819
21. Jarrett, J. T., Berger, E. P., and Lansbury, P. T., Jr. (1993) The carboxyl terminus of the β-amyloid protein is critical for the seeding of amyloid formation. Implications for the pathogenesis of Alzheimers disease. *Biochemistry* **32,** 4693–4697
22. Furukawa, Y., Kaneko, K., Matsumoto, G., Kurosawa, M., and Nukina, N. (2009) Cross-seeding fibrillation of Q/N-rich proteins offers new pathomechanism of polyglutamine diseases. *J. Neurosci*. **29,** 5153–5162
23. Lundmark, K., Westermark, G. T., Olsén, A., and Westermark, P. (2005) Protein fibrils in nature can enhance amyloid protein A amyloidosis in mice. Cross-seeding as a disease mechanism. *Proc. Natl. Acad. Sci. U.S.A.* **102,** 6098–6102
24. Morales, R., Estrada, L. D., Diaz-Espinoza, R., Morales-Scheihing, D., Jara, M. C., Castilla, J., and Soto, C. (2010) Molecular cross-talk between misfolded proteins in animal models of Alzheimers and prion diseases. *J. Neurosci.* **30,** 4528–4535
25. O'Nuallain, B., Williams, A. D., Westermark, P., and Wetzel, R. (2004) Seeding specificity in amyloid growth induced by heterologous fibrils. *J. Biol. Chem.* **279,** 17490–17499
26. Götz, J., Chen, F., van Dorpe, J., and Nitsch, R. M. (2001) Formation of neurofibrillary tangles in P301l tau transgenic mice induced by Aβ42 fibrils. *Science* **293,** 1491–1495
27. Lasagna-Reeves, C. A., Castillo-Carranza, D. L., Guerrero-Muoz, M. J., Jackson, G. R., and Kayed, R. (2010) Preparation and characterization of neurotoxic tau oligomers. *Biochemistry* **49,** 10039–10041
28. Derkatch, I. L., Uptain, S. M., Outeiro, T. F., Krishnan, R., Lindquist, S. L., and Liebman, S. W. (2004) Effects of Q/N-rich, poly(Q), and nonpoly(Q) amyloids on the *de novo* formation of the [PSI$^+$] prion in yeast and aggregation of Sup35 *in vitro*. *Proc. Natl. Acad. Sci. U.S.A.* **101,** 12934–12939
29. Vanik, D. L., Surewicz, K. A., and Surewicz, W. K. (2004) Molecular basis of barriers for interspecies transmissibility of mammalian prions. *Mol. Cell* **14,** 139–145
30. Kocisko, D. A., Priola, S. A., Raymond, G. J., Chesebro, B., Lansbury, P. T., Jr., and Caughey, B. (1995) Species specificity in the cell-free conversion of prion protein to protease-resistant forms. A model for the scrapie species barrier. *Proc. Natl. Acad. Sci. U.S.A.* **92,** 3923–3927
31. Prusiner, S. B., Scott, M., Foster, D., Pan, K. M., Groth, D., Mirenda, C., Torchia, M., Yang, S. L., Serban, D., Carlson, G. A., Hoppe, P. C., Westaway, D., and Dearmond, S. J. (1990) Transgenetic studies implicate interactions between homologous Prp isoforms in scrapie prion replication. *Cell* **63,** 673–686
32. Chen, B., Newnam, G. P., and Chernoff, Y. O. (2007) Prion species barrier between the closely related yeast proteins is detected despite coaggregation. *Proc. Natl. Acad. Sci. U.S.A.* **104,** 2791–2796
33. Santoso, A., Chien, P., Osherovich, L. Z., and Weissman, J. S. (2000) Molecular basis of a yeast prion species barrier. *Cell* **100,** 277–288
34. DePace, A. H., Santoso, A., Hillner, P., and Weissman, J. S. (1998) A critical role for amino-terminal glutamine/asparagine repeats in the formation and propagation of a yeast prion. *Cell* **93,** 1241–1252
35. Tanaka, M., Chien, P., Yonekura, K., and Weissman, J. S. (2005) Mechanism of cross-species prion transmission. An infectious conformation compatible with two highly divergent yeast prion proteins. *Cell* **121,** 49–62
36. Biere, A. L., Wood, S. J., Wypych, J., Steavenson, S., Jiang, Y., Anafi, D., Jacobsen, F. W., Jarosinski, M. A., Wu, G. M., Louis, J. C., Martin, F., Narhi, L. O., and Citron, M. (2000) Parkinson disease-associated α-synuclein is more fibrillogenic than β- and γ-synuclein and cannot cross-seed its homologs. *J. Biol. Chem.* **275,** 34574–34579
37. Wright, C. F., Teichmann, S. A., Clarke, J., and Dobson, C. M. (2005) The importance of sequence diversity in the aggregation and evolution of proteins. *Nature* **438,** 878–881
38. Krebs, M. R., Morozova-Roche, L. A., Daniel, K., Robinson, C. V., and Dobson, C. M. (2004) Observation of sequence specificity in the seeding of protein amyloid fibrils. *Protein Sci.* **13,** 1933–1938
39. Austin, J. W., Sanders, G., Kay, W. W., and Collinson, S. K. (1998) Thin aggregative fimbriae enhance *Salmonella enteritidis* biofilm formation. *FEMS Microbiol. Lett.* **162,** 295–301
40. Dueholm, M. S., Petersen, S. V., Sonderkaer, M., Larsen, P., Christiansen, G., Hein, K. L., Enghild, J. J., Nielsen, J. L., Nielsen, K. L., Nielsen, P. H., and Otzen, D. E. (2010) Functional amyloid in *Pseudomonas*. *Mol. Microbiol.* **77,** 1009–1020
41. Elliot, M. A., Karoonuthaisiri, N., Huang, J., Bibb, M. J., Cohen, S. N., Kao, C. M., and Buttner, M. J. (2003) The chaplins. A family of hydrophobic cell-surface proteins involved in aerial mycelium formation in *Streptomyces coelicolor*. *Genes Dev.* **17,** 1727–1740
42. Olsén, A., Jonsson, A., and Normark, S. (1989) Fibronectin binding mediated by a novel class of surface organelles on *Escherichia coli*. *Nature* **338,** 652–655
43. Kikuchi, T., Mizunoe, Y., Takade, A., Naito, S., and Yoshida, S. (2005) Curli fibers are required for development of biofilm architecture in *Escherichia coli* K-12 and enhance bacterial adherence to human uroepithelial cells. *Microbiol. Immunol.* **49,** 875–884
44. Prigent-Combaret, C., Prensier, G., Le Thi, T. T., Vidal, O., Lejeune, P., and Dorel, C. (2000) Developmental pathway for biofilm formation in curli-producing *Escherichia coli* strains. Role of flagella, curli, and colanic acid. *Environ. Microbiol.* **2,** 450–464
45. Weiss-Muszkat, M., Shakh, D., Zhou, Y., Pinto, R., Belausov, E., Chapman, M. R., and Sela, S. (2010) Biofilm formation by and multicellular behavior of *Escherichia coli* O55:H7, an atypical enteropathogenic strain. *Appl. Environ. Microbiol.* **76,** 1545–1554
46. Tukel, C., Nishimori, J. H., Wilson, R. P., Winter, M. G., Keestra, A. M., van Putten, J. P., and Baumler, A. J. (2010) Toll-like receptors 1 and 2 cooperatively mediate immune responses to curli, a common amyloid from enterobacterial biofilms. *Cell Microbiol.* **12,** 1495–1505
47. Tükel, C., Wilson, R. P., Nishimori, J. H., Pezeshki, M., Chromy, B. A., and Bäumler, A. J. (2009) Responses to amyloids of microbial and host origin are mediated through Toll-like receptor 2. *Cell Host Microbe* **6,** 45–53

48. Tükel, C., Raffatellu, M., Humphries, A. D., Wilson, R. P., Andrews-Polymenis, H. L., Gull, T., Figueiredo, J. F., Wong, M. H., Michelsen, K. S., Akçelik, M., Adams, L. G., Bäumler, A. J. (2005) CsgA is a pathogen-associated molecular pattern of *Salmonella enterica* serotype *Typhimurium* that is recognized by Toll-like receptor 2. *Mol. Microbiol.* **58,** 289–304
49. Barnhart, M. M., and Chapman, M. R. (2006) Curli biogenesis and function. *Annu. Rev. Microbiol.* **60,** 131–147
50. Gerstel, U., and Römling, U. (2003) The csgD promoter, a control unit for biofilm formation in *Salmonella typhimurium*. *Res. Microbiol.* **154,** 659–667
51. Hammer, N. D., Schmidt, J. C., and Chapman, M. R. (2007) The curli nucleator protein, CsgB, contains an amyloidogenic domain that directs CsgA polymerization. *Proc. Natl. Acad. Sci. U.S.A.* **104,** 12494–12499
52. Robinson, L. S., Ashman, E. M., Hultgren, S. J., and Chapman, M. R. (2006) Secretion of curli fiber subunits is mediated by the outer membrane-localized CsgG protein. *Mol. Microbiol.* **59,** 870–881
53. Nenninger, A. A., Robinson, L. S., Hammer, N. D., Epstein, E. A., Badtke, M. P., Hultgren, S. J., and Chapman, M. R. (2011) CsgE is a curli secretion specificity factor that prevents amyloid fiber aggregation. *Mol. Microbiol.* **81,** 486–499
54. Nenninger, A. A., Robinson, L. S., and Hultgren, S. J. (2009) Localized and efficient curli nucleation requires the chaperone-like amyloid assembly protein CsgF. *Proc. Natl. Acad. Sci. U.S.A.* **106,** 900–905
55. Wang, X., Smith, D. R., Jones, J. W., and Chapman, M. R. (2007) *In vitro* polymerization of a functional *Escherichia coli* amyloid protein. *J. Biol. Chem.* **282,** 3713–3719
56. Datsenko, K. A., and Wanner, B. L. (2000) One-step inactivation of chromosomal genes in *Escherichia coli* K-12 using PCR products. *Proc. Natl. Acad. Sci. U.S.A.* **97,** 6640–6645
57. Cegelski, L., Pinkner, J. S., Hammer, N. D., Cusumano, C. K., Hung, C. S., Chorell, E., Aberg, V., Walker, J. N., Seed, P. C., Almqvist, F., Chapman, M. R., and Hultgren, S. J. (2009) Small-molecule inhibitors target *Escherichia coli* amyloid biogenesis and biofilm formation. *Nat. Chem. Biol.* **5,** 913–919
58. Wang, X., Zhou, Y., Ren, J. J., Hammer, N. D., and Chapman, M. R. (2010) Gatekeeper residues in the major curlin subunit modulate bacterial amyloid fiber biogenesis. *Proc. Natl. Acad. Sci. U.S.A.* **107,** 163–168
59. White, A. P., Gibson, D. L., Collinson, S. K., Banser, P. A., and Kay, W. W. (2003) Extracellular polysaccharides associated with thin aggregative fimbriae of *Salmonella enterica* serovar *enteritidis*. *J. Bacteriol.* **185,** 5398–5407
60. Malone, C. L., Boles, B. R., Lauderdale, K. J., Thoendel, M., Kavanaugh, J. S., and Horswill, A. R. (2009) Fluorescent reporters for *Staphylococcus aureus*. *J. Microbiol. Methods* **77,** 251–260
61. Hammer, N. D., McGuffie, B. A., Zhou, Y., Badtke, M. P., Reinke, A. A., Brännström, K., Gestwicki, J. E., Olofsson, A., Almqvist, F., and Chapman, M. R. (2012) The C-terminal repeating units of CsgB direct bacterial functional amyloid nucleation. *J. Mol. Biol.* **422,** 376–389
62. Morten, S., Dueholm, P. H. N., Chapman, M., and Otzen, D. (2012) *Functional Amyloid in Bacteria*, Wiley, New York, in press
63. Wang, X., Hammer, N. D., and Chapman, M. R. (2008) The molecular basis of functional bacterial amyloid polymerization and nucleation. *J. Biol. Chem.* **283,** 21530–21539
64. Wang, X., and Chapman, M. R. (2008) Sequence determinants of bacterial amyloid formation. *J. Mol. Biol.* **380,** 570–580
65. Zogaj, X., Bokranz, W., Nimtz, M., and Römling, U. (2003) Production of cellulose and curli fimbriae by members of the family Enterobacteriaceae isolated from the human gastrointestinal tract. *Infect. Immun.* **71,** 4151–4158
66. Römling, U. (2005) Characterization of the rdar morphotype, a multicellular behavior in Enterobacteriaceae. *Cell Mol. Life Sci.* **62,** 1234–1246
67. Römling, U., Bian, Z., Hammar, M., Sierralta, W. D., and Normark, S. (1998) Curli fibers are highly conserved between *Salmonella typhimurium* and *Escherichia coli* with respect to operon structure and regulation. *J. Bacteriol.* **180,** 722–731
68. Kai-Larsen, Y., Lüthje, P., Chromek, M., Peters, V., Wang, X., Holm, A., Kádas, L., Hedlund, K. O., Johansson, J., Chapman, M. R., Jacobson, S. H., Römling, U., Agerberth, B., and Brauner, A. (2010) Uropathogenic *Escherichia coli* modulates immune responses and its curli fimbriae interact with the antimicrobial peptide LL-37. *PLoS Pathog.* **6,** e1001010
69. Jarrett, J. T., and L. P. J. (1993) Seeding "one-dimensional crystallization" of amyloid. A pathogenic mechanism in Alzheimer disease and scrapie? *Cell* **18,** 1055–1058
70. Shu, Q., Crick, S. L., Pinkner, J. S., Ford, B., Hultgren, S. J., and Frieden, C. (2012) The *E. coli* CsgB nucleator of curli assembles to β-sheet oligomers that alter the CsgA fibrillization mechanism. *Proc. Natl. Acad. Sci. U.S.A.* **109,** 6502–6507
71. White, A. P., Collinson, S. K., Banser, P. A., Gibson, D. L., Paetzel, M., Strynadka, N. C., and Kay, W. W. (2001) Structure and characterization of AgfB from *Salmonella enteritidis* thin aggregative fimbriae. *J. Mol. Biol.* **311,** 735–749
72. Chien, P., and Weissman, J. S. (2001) Conformational diversity in a yeast prion dictates its seeding specificity. *Nature* **410,** 223–227
73. Jones, E. M., and Surewicz, W. K. (2005) Fibril conformation as the basis of species- and strain-dependent seeding specificity of mammalian prion amyloids. *Cell* **121,** 63–72
74. Ma, B., and Nussinov, R. (2012) Selective molecular recognition in amyloid growth and transmission and cross-species barriers. *J. Mol. Biol.* **421,** 172–184
75. Tanaka, M., Chien, P., Naber, N., Cooke, R., and Weissman, J. S. (2004) Conformational variations in an infectious protein determine prion strain differences. *Nature* **428,** 323–328
76. Tessier, P. M., and Lindquist, S. (2007) Prion recognition elements govern nucleation, strain specificity, and species barriers. *Nature* **447,** 556–561
77. Nelson, R., Sawaya, M. R., Balbirnie, M., Madsen, A. Ø., Riekel, C., Grothe, R., and Eisenberg, D. (2005) Structure of the cross-β spine of amyloid-like fibrils. *Nature* **435,** 773–778
78. Lipfert, J., Franklin, J., Wu, F., and Doniach, S. (2005) Protein misfolding and amyloid formation for the peptide GNNQQNY from yeast prion protein Sup35. Simulation by reaction path annealing. *J. Mol. Biol.* **349,** 648–658
79. Kim, S. M., Lee, H. W., Choi, Y. W., Kim, S. H., Lee, J. C., Lee, Y. C., Seol, S. Y., Cho, D. T., and Kim, J. (2012) Involvement of curli fimbriae in the biofilm formation of *Enterobacter cloacae*. *J. Microbiol.* **50,** 175–178
80. Gudmundsson, G. H., Agerberth, B., Odeberg, J., Bergman, T., Olsson, B., and Salcedo, R. (1996) The human gene *FALL39* and processing of the cathelin precursor to the antibacterial peptide LL-37 in granulocytes. *Eur. J. Biochem.* **238,** 325–332
81. Frohm Nilsson, M., Sandstedt, B., Sørensen, O., Weber, G., Borregaard, N., and Ståhle-Bäckdahl, M. (1999) The human cationic antimicrobial protein (hCAP18), a peptide antibiotic, is widely expressed in human squamous epithelia and colocalizes with interleukin-6. *Infect. Immun.* **67,** 2561–2566
82. Kobayashi, H., Tada, S., Fuchigami, T., Okuda, Y., Takasugi, K., Matsumoto, T., Iida, M., Aoyagi, K., Iwashita, A., Daimaru, Y., and Fujishima, M. (1996) Secondary amyloidosis in patients with rheumatoid arthritis. Diagnostic and prognostic value of gastroduodenal biopsy. *Br J. Rheumatol.* **35,** 44–49
83. Rickard, A. H., Gilbert, P., High, N. J., Kolenbrander, P. E., and Handley, P. S. (2003) Bacterial coaggregation. An integral process in the development of multispecies biofilms. *Trends Microbiol.* **11,** 94–100
84. Peters, B. M., Jabra-Rizk, M. A., O'May, G. A., Costerton, J. W., and Shirtliff, M. E. (2012) Polymicrobial interactions. Impact on pathogenesis and human disease. *Clin. Microbiol. Rev.* **25,** 193–213
85. Jordal, P. B., Dueholm, M. S., Larsen, P., Petersen, S. V., Enghild, J. J., Christiansen, G., Hojrup, P., Nielsen, P. H., and Otzen, D. E. (2009) Widespread abundance of functional bacterial amyloid in mycolata and other Gram-positive bacteria. *Appl. Environ. Microb.* **75,** 4101–4110