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Review

Bacterial Amyloid Formation: Structural Insights into Curli Biogensis

Nani Van Gerven,[1,2] Roger D. Klein,[3] Scott J. Hultgren,[3] and Han Remaut[1,2,*]

Curli are functional amyloid fibers assembled by many Gram-negative bacteria as part of an extracellular matrix that encapsulates the bacteria within a biofilm. A multicomponent secretion system ensures the safe transport of the aggregation-prone curli subunits across the periplasm and outer membrane, and coordinates subunit self-assembly into surface-attached fibers. To avoid the build-up of potentially toxic intracellular protein aggregates, the timing and location of the interactions of the different curli proteins are of paramount importance. Here we review the structural and molecular biology of curli biogenesis, with a focus on the recent breakthroughs in our understanding of subunit chaperoning and secretion. The mechanistic insight into the curli assembly pathway will provide tools for new biotechnological applications and inform the design of targeted inhibitors of amyloid polymerization and biofilm formation.

Biofilm Formation

To aid colonization and persistence in host or environmental niches, many bacteria coalesce to form encapsulated communities embedded within a complex hydrated matrix of proteins, nucleic acids, and polysaccharides. These communities, known as biofilms, protect the bacteria within from physical and chemical stresses, such as oxidative damage and desiccation [1,2]. The build-up of these biofilms on household, industrial, and medical equipment results in a potential reservoir of infectious agents recalcitrant to traditional cleaning techniques. Further, biofilm formation by pathogenic bacterial strains within hosts drastically reduces their susceptibility to host immune responses and therapeutic antimicrobial agents, and constitutes a major health concern [3–10].

Biofilm formation is composed of a series of related steps. Following the reversible cell-surface and/or cell–cell adherence of planktonic microoganisms, filamentous structures known as pili mediate a robust surface attachment and inclusion in an extracellular matrix [11]. In *Escherichia coli* and *Salmonella enterica* biofilms, aggregative fibers known as curli (sometimes referred to by their now-obsolete name, tafi) constitute the major proteinaceous component of this extracellular matrix [12]. These matrices promote the formation of floating biofilms (pellicles) at the air–liquid interface of static liquid cultures and can mediate the adhesion of solid cultures to biotic and abiotic surfaces, such as animal and plant tissue, stainless steel, and glass [13–20].

Curli fibers are produced by a dedicated secretion pathway known as the nucleation-precipitation mechanism, or the type VIII secretion system [21,22]. In *E. coli*, seven curli-specific genes (*csg*) make up the structural components and assembly apparatus of the curli fibers and are encoded by two divergently transcribed operons (*csgBAC* and *csgDEFG*), respectively [21]

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CsgC acts as a chaperone to prevent or neutralize premature, periplasmic amyloidosis of curli subunits. The protein does so at low substoichiometric ratios and in the absence of ATP.

The CsgG lipoprotein forms a composite transmembrane β-barrel. Transition from a soluble prepore to the outer membrane-bound pore conformation entails nonamerization and extension of two adjacent β-hairpins per subunit.

The CsgG nonamer forms a constitutive peptide diffusion channel that cooperates with CsgE to expel curli subunits in an entropy-driven process.

CsgE forms a periplasmic, nonameric secretion adaptor that binds and caps a preconstriction chamber in the curli transporter CsgG.

CsgF acts as a CsgG-bound curli assembly factor that coordinates the function of the curli nucleator CsgB with CsgG's secretion of the major curli subunit CsgA.

[1]Structural and Molecular Microbiology, Structural Biology Research Center, VIB, Pleinlaan 2, 1050 Brussels, Belgium
[2]Structural Biology Brussels, Vrije Universiteit Brussel, Pleinlaan 2, 1050 Brussels, Belgium
[3]Department of Molecular Microbiology and Microbial Pathogenesis, Washington University in Saint Louis School of Medicine, St Louis, MO 63110-1010, USA

*Correspondence:
han.remaut@vib-vub.be (H. Remaut).

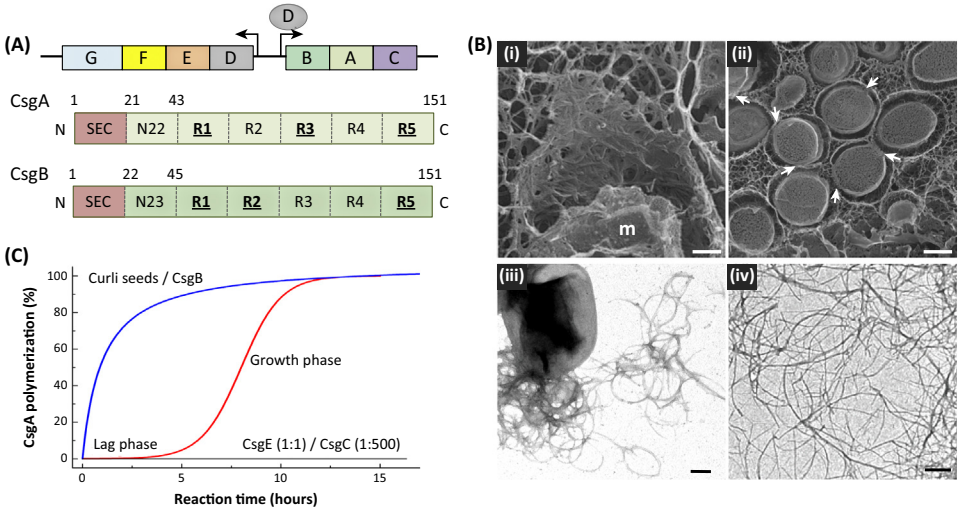

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Figure 1. Curli Composition and Structure. (A) Organization of the *csgBAC* and *csgDEFG* curli operons and architecture of the curli subunits CsgA (light green) and CsgB (dark green). The N-terminal signal sequence (SEC; red) is cleaved after export into the periplasm. The mature subunits contain an N-terminal curli-specific targeting sequence (N22 or N23 in CsgA and CsgB, respectively) that is followed by a pseudo-repeat region (R1 to R5) that forms the amyloidogenic core of the curli subunits (green). Repeats that efficiently self-polymerize *in vitro* are underscored. (B) Electron microscopy of curli fibers. (i, ii) Freeze-fracture electron microscopy of *Escherichia coli* biofilms shows that bacterial cells are encased in a matrix supported by interwoven curli. Bacteria appear to come into contact with the matrix only at discrete locations (white arrows); (m: fractioned bacterial membrane); scale bars: 100 nm (i) and 500 nm (ii) (Reproduced from [12]). (iii, iv) Transmission electron microscopy of individual *E. coli* cells producing curli fibers (iii), and curli-like fibers grown *in vitro* from purified CsgA (iv); scale bars: 200 nm. (C) Representation of typical *in vitro* CsgA polymerization profiles under different conditions. The addition of preformed fibers or the CsgB nucleator removes the lag phase preceding exponential fiber growth (blue curve). In the presence of CsgE (1:1 ratio) or CsgC (1:500 ratio), no CsgA polymerization is observed (black curve) [24].

(Figure 1A). Two recent papers in the field by Goyal *et al.* (2014) and Evans *et al.* (2015) have greatly advanced our understanding of the structural components of curli transport and secretion [23,24]. In this work, we review the structural and mechanistic aspects of curli fiber structure and assembly, and the applicability of these exciting findings to the study of bacterial biofilms and human pathogenic amyloids.

Curli Fibers

Upon visualization by electron microscopy, *E. coli*-associated curli usually appear as a tangled mass of linear, surface-associated fibers of 4–6 nm width and several micrometers length (Figure 1B). Within biofilms, curli fibers form an interwoven mesh that supports the extracellular matrix and encapsulates individual cells (Figure 1B). Solid-state nuclear magnetic resonance (NMR) experiments have demonstrated that curli fibers make up roughly 85% of the matrix material in curli-associated biofilms [12]. In these matrices, curli frequently appear detached from the embedded cells. It is unclear if this observation represents a regulated process necessary for efficient biofilm matrix formation, or is an artefact of cell desiccation during sample preparation procedures.

Curli are noncovalent heteropolymeric filaments of CsgA and CsgB subunits, present at ratios of approximately 20:1 CsgA:CsgB in *in vivo* wild-type fibers [25]. Curli belong to a class of stable, ordered protein aggregates known as amyloids [21,26]. Although commonly associated with pathological protein misfolding in human diseases [27–30], a significant body of research now suggests that amyloids are also intentionally produced by a variety of organisms to fulfil important physiological functions, such as regulation of hydrophobicity during fungal