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Bacterial synthase-dependent exopolysaccharide secretion: a focus on cellulose

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Bacterial biofilms are a prevalent multicellular life form in which individual members can undergo significant functional differentiation and are typically embedded in a complex extracellular matrix of proteinaceous fimbriae, extracellular DNA, and exopolysaccharides (EPS). Bacteria have evolved at least four major mechanisms for EPS biosynthesis, of which the synthase-dependent systems for bacterial cellulose secretion (Bcs) represent not only key biofilm determinants in a wide array of environmental and host-associated microbes, but also an important model system for the studies of processive glycan polymerization, cyclic diguanylate (c-di-GMP)-dependent synthase regulation, and biotechnological polymer applications. The secreted cellulosic chains can be decorated with additional chemical groups or can pack with various degrees of crystallinity depending on dedicated enzymatic complexes and/or cytoskeletal scaffolds. Here, I review recent progress in our understanding of synthase-dependent EPS biogenesis with a focus on common and idiosyncratic molecular mechanisms across diverse cellulose secretion systems.

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Introduction

Whether in abiotic environments or associated with eukaryotic hosts, bacteria typically live in close quarters as mono- or mixed-species multicellular communities called biofilms. A key feature of the latter is spatial differentiation, where subsets of members are designated to secrete extracellular matrix components — such as extracellular DNA (eDNA), proteinaceous fimbriae (e.g. cell-surface adhesins or amyloid curli), and/or exopolysaccharides (EPS) — whereas others take charge of cell proliferation and biofilm dispersal [1–3]. EPS often provide the structural scaffold of biofilm macrocolonies and partake in redox control, biofilm hydration, nutrient exchange, surface colonization, and immune escape within the host. Bacteria have evolved a remarkable variety of systems for EPS and glycoconjugate biogenesis, which can be viewed as variations of four major mechanisms [4–7]. Apart from surface-associated sucrases, which generate dextrans or levans from hydrolyzed extracellular sucrose, the three other pathways — ATP-binding cassette (ABC) transporter-, Wzx/Wzy-, and synthase-dependent — rely on intracellular precursor polymerization and multicomponent assemblies for the export and modifications of the polymeric products [4–7].

Both ABC transporter- and Wzx (flippase)/Wzy (polymerase)-dependent systems involve the synthesis of isoprenoid- or phospholipid-linked polysaccharides or oligosaccharidic modules that are exported across the inner membrane (IM) using the energy of coupled ATP hydrolysis cycles or the countermovement of protons, respectively. ABC transporter-dependent pathways involve the biosynthesis of some lipopolysaccharide (LPS) O-antigens, isoprenoid-linked glycans for protein N-glycosylation, phosphatidylglycerol-linked capsular polysaccharides (CPS) in Gram-negative mucosal pathogens, and cell wall teichoic acids in Gram-positive bacteria [4–7]. Many O-antigen LPS are alternatively synthesized by Wzx/Wzy-like flippase-dependent systems and similar pathways ensure the biogenesis of diverse capsular and biofilm polysaccharides in both Gram-positive and Gram-negative bacteria (e.g. *Streptococcus pneumoniae* CPS, *Xanthomonas campestris* xanthan and *Xylella fastidiosa* fastidian gums, *Pseudomonas aeruginosa* Psl and *Vibrio cholerae* Vps, enterobacterial colanic acids, etc.) [4–7].

In contrast to the above, most synthase-dependent systems do not require lipid-linked initial acceptors and use highly processive polymerase/copolymerase tandems to couple linear glycan polymerization with IM translocation [4,7,8]. Examples include the alginate, poly-N-acetylglucosamine (PNAG) and Pel EPS (e.g. in *P.*

aeruginosa), bacterial hyaluronic acid (e.g. in *Streptococcus pyogenes*), and cellulose secretion systems, encoded by dedicated operons or gene clusters with high mosaicity [4,7–10].

Over the last few years, an impressive anthology of resolved structures, mechanistic idiosyncrasies, and commonalities among synthase-dependent EPS secretion systems has been reported in the literature, whereas comparative genomics and modeling studies allow to identify and predict functionalities of homologous systems in an ever-growing catalog of microbes of interest. This mini-review highlights key aspects of these impressive machineries with focus on the widespread and intrinsically diverse bacterial cellulose secretion (Bcs) systems.

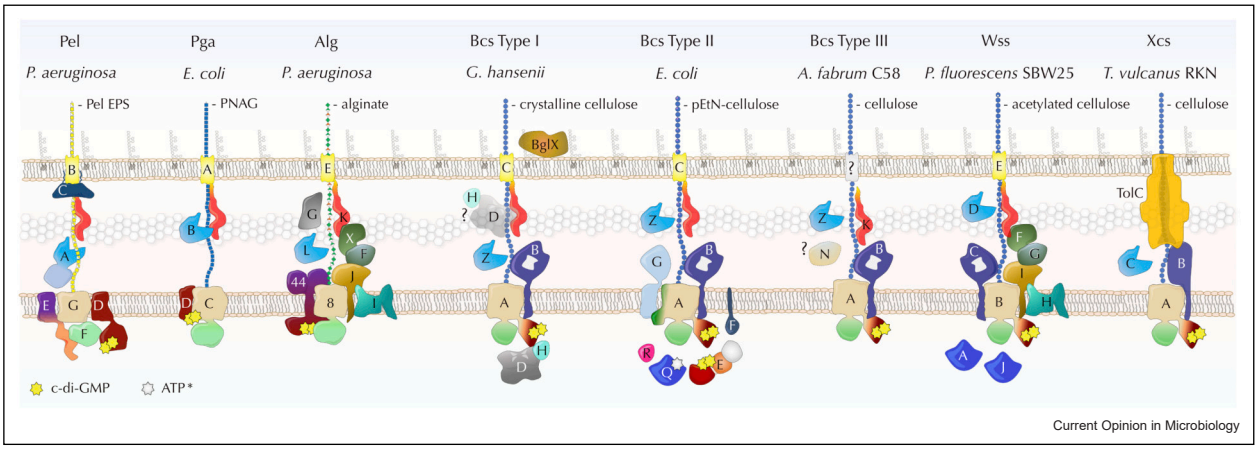
Cellulose synthases: activity and regulation

Glycan polymerization and IM translocation in synthase-dependent systems can be carried out by separate proteins (e.g. PelF and PelG, respectively, in Pel systems), or be incorporated in a single subunit as is the case with BcsA, Alg8, and PgaC in cellulose, alginate, and PNAG biogenesis [7,8,11]. Importantly, EPS synthases are likely to function as parts of macromolecular trans-envelope complexes, where similar functionalities have evolved across multiple different systems, often despite a lack of sequence or fold conservation across the respective subunits [7,8,11]. These include tight association with an IM copolymerase necessary for the activation and/or stability of the catalytic subunit (e.g. PelE, BcsB, Alg44, and PgaD); c-di-GMP-sensing protein modules for system activation (e.g. PilZ domains on BcsA and Alg44, I-site motifs on degenerate [*] GGDEF domains from BcsE and PelD, and a composite binding site at the PgaC and PgaD interface); tetratricopeptide repeat (TPR)-rich periplasmic scaffolds (e.g. AlgK and periplasmic modules of BcsC, PelB, and PgaA); polymer-modification (e.g. alginate and cellulose acetylation complexes) and hydrolase/lyase enzymes (e.g. AlgL, BcsZ, PelA, and PgaB) in the periplasm; and β-barrel export modules in the outer membrane (AlgE and porin domains from PelB, BcsC, and PgaA) [7,8,11] (Figure 1).

Dependent on encoded accessory subunits discussed below, Bcs systems have been grouped into several major types: Type-I systems characterized by the presence of the BcsD scaffolding subunit; Type-II or *E. coli*-like systems encoding the BcsE c-di-GMP-sensing and BcsG phosphoethanolamine (pEtN)-transferase subunits; Type-III systems, which lack all three of the above and often feature BcsK instead of BcsC in the periplasm; Wss systems for acetylated cellulose secretion; and hybrid systems combining features from the above [7,10] (Figure 1).

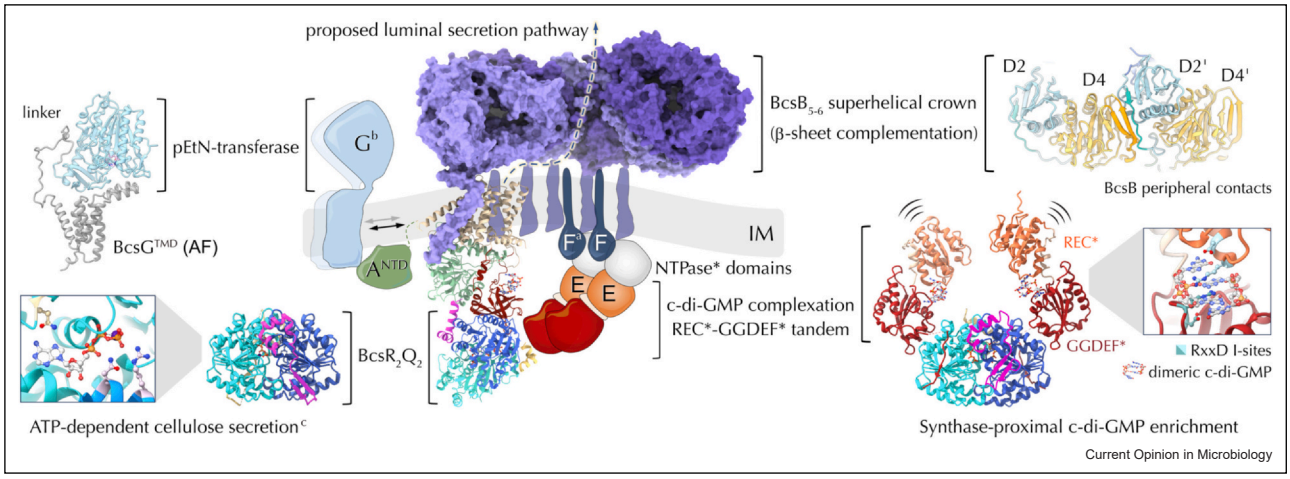
BcsA's core for EPS polymerization and export is conserved from bacteria to higher plants and consists of an α-helical transmembrane domain (TMD) interrupted by a D,D,D,Q(Q/R)xRW-type glycosyltransferase (GT) domain from the GT2 family of enzymes, and a so-called gating loop that caps the active site pocket [7,12,13]. The last tryptophan from the Q(Q/R)xRW motif coordinates the terminal glycosyl moiety of the nascent EPS at the active site and two of the conserved spaced aspartates (D^{246}xD in *R. sphaeroides* BcsA) coordinate the substrate's diphosphate via a divalent metal ion. The third aspartate (D^{343} in *R. sphaeroides* BcsA) serves as the catalytic base and lies at the tip of the so-called 'finger helix,' whose minute movements aid polymer

Figure 1



Major types of synthase-dependent EPS secretion systems in Gram-negative bacteria. *, ATP binding and hydrolysis are likely involved in Bcs system assembly and not in glucose polymerization and cellulose extrusion.
Updated and expanded from reference [7] (http://creativecommons.org/licenses/by-nc/4.0/).

Figure 2



Structure–function insights into the pEtN-cellulose secretion system. TMD, transmembrane domain; AF, alphafold2 model; NTPase*, degenerate, that is, catalytically incompetent, NTPase-like domain; REC*, degenerate, that is, phosphorylation-incompetent, receiver domain; GGDEF*, degenerate, that is, diguanylate cyclase-incompetent, GGDEF domain; I-site, an RxxD c-di-GMP-binding motif found on diguanylate cyclase domains as an inhibitory or diguanylate signal relay motif; D, domain. Structural data from references [18,20,31,33,40]. **(a)** a dimeric BcsF stoichiometry has not been directly visualized but is consistent with the protein's running behavior in denaturing gel electrophoresis, bacterial two-hybrid (BACTH) assays of protein interactions, and the BcsF-dependent recruitment of BcsE to the membrane (BcsE is dimeric both in the soluble Bcs-ERQ complex and the membrane-embedded Bcs macrocomplex). **(b)** BACTH experiments have shown that BcsG interacts with the *E. coli*-specific $BcsA^{NTD}$ domain, whereas tubular densities in the detergent micelle have been interpreted as two copies of $BcsG^{NTD}$ in a low-resolution cryo-EM study of the Bcs macrocomplex. **(c)** ATP hydrolysis is not required for synthase activity in vitro, however, BcsRQ mutation analyses indicate that both ATP binding and hydrolysis might be necessary for cellulose biogenesis in vivo. In particular, *bcsQ* mutations disrupting ATP complexation (e.g. $BcsQ^{N152A–R156A–N171A}$) lead to loss of cellulose secretion, mutations stimulating BcsRQ ATPase activity (e.g. $BcsQ^{R156E}$) lead to enhanced cellulose secretion, and mutations that lead to a stable, ATP-binding, but catalytically inactive BcsRQ complex (e.g. $BcsQ^{T15K}$) lead again to loss of cellulose secretion, thus excluding a purely structural role for nucleotide complexation.

translocation [7,12,13]. In bacteria, the cellulose synthase contains a third, PilZ domain, whose core-adjacent linker carries the RxxxR motif for dimeric cyclic diguanylate (c-di-GMP) coordination [7,13,14]. Dynamic dinucleotide binding causes gating loop relaxation and together with the 'finger helix' regulates the reiterative processes of uridine diphosphate (UDP) product release, polymer translocation, UDP-glucose substrate entry, gating loop closure, and catalysis [7,13].

Whereas the catalytic BcsA cycle has been characterized primarily *in crystallo* in saturating concentrations of dinucleotide [14], product, and/or substrate-like ligands, in nature, multiple mechanisms have evolved for the local enrichment of activating c-di-GMP. *E. coli*, for example, resorts to one or more dedicated diguanylate cyclases such as synthase-interacting AdrA/DgcA in some strains or YedQ/DgcQ in others [15,16]. In addition, *E. coli* BcsA forms a stable, multicomponent biosynthetic macrocomplex encompassing most of the inner-membrane and cytosolic subunits ($BcsR_2Q_2AB_{5–6}E_2F_2$) (Figure 2). In it, the essential-for-secretion BcsRQ tandem has been proposed to regulate macrocomplex assembly in an ATP-dependent manner and to contribute to BcsA maintenance and catalytic activity in the membrane [17,18]. The BcsE protein, on the other hand, features a tripartite architecture of catalytically or phosphotransfer-incompetent NTPase (NTPase*), receiver (REC*), and diguanylate cyclase (GGDEF*) domains. Dimeric BcsE is recruited by BcsF to the membrane, where each BcsE copy presents a composite site for dimeric c-di-GMP coordination formed by two RxxD motifs on its degenerate receiver and diguanylate cyclase domains ($BcsE^{REC*-GGDEF*}$) [19,20] and, together with the BcsRQ subunits, BcsE forms a c-di-GMP-binding cytosolic vestibule around BcsA's PilZ domain [17,18] (Figure 2). Changes in the contribution of either RxxD motif have been shown to alter the BcsE's affinity for c-di-GMP and its observed dynamic structure — evidenced in both crystallographic and cryo-EM experiments — has been proposed to act as a dynamic partner for dinucleotide recycling in processive synthase activation [7,18,20].

As mentioned above, BcsA functions in tandem with the BcsB copolymerase [7]. The latter features a donut-shaped periplasmic module that incorporates 2 carbohydrate-binding and 2 flavodoxin-like domains alternating along the polypeptide chain, and a C-proximal membrane anchor that is required for BcsA's catalytic function and is composed of a short amphipathic and a transmembrane α-helices [12,18,21]. Crystal structures

of the *Rhodobacter sphaeroides* BcsAB tandem have revealed a 1:1 complex where the C-terminal BcsB anchor helix completes BcsA's export domain [12] and a similar 1:1 architecture was observed in a low-resolution negative-stain electron microscopy reconstruction of a heterodimeric *G. hansenii* BcsAB tandem [22], itself encoded by a fused *bcsAB* gene and further supporting equimolar BcsA:BcsB assembly [7,10].

Surprisingly, the cryo-EM structure of the *E. coli* Bcs macrocomplex, revealed a drastically different, non-canonical BcsA:BcsB stoichiometry [18]. In it, a single BcsA subunit assembles with up to 6 BcsB protomers that use a β-sheet complementation mechanism to form a superhelical 'crown' in the periplasm, with cellulose proposed to glide outward along stacked luminal loops from BcsB's carbohydrate-binding domains [18] (Figure 2). Importantly, the superhelical assembly of the periplasmic modules and the presence of the transmembrane BcsB tail anchors likely induce and favor significant negative curvature in the membrane [18] (Figure 2). Interestingly, visualization of the assembly ATPase BcsQ in cells and of secreted cellulose in biofilm cryosections is consistent with elongated cell shape and polar pEtN-cellulose secretion in the younger biofilm layers (i.e. Bcs targeting to membrane domains with the highest negative curvature), whereas the oldest surface layers present rounded, nondividing cells fully enclosed in a mesh of pEtN-cellulose and curli [23,24]. Whether subcellular targeting of the Bcs system can be achieved by covarying cell shape and BcsA:BcsB stoichiometry remains to be experimentally examined.

Cellulosic homo- and heteropolymers

Although some bacterial EPS-producing synthases can yield mixed-linkage glycans [25], most of these enzymes typically use a specific nucleotide sugar donor to transfer the glycosyl moiety via a unique glycosidic linkage onto a processively extruded polymer. Nevertheless, this more prevalent "one enzyme–one substrate–one linkage" principle does not necessarily translate into the secretion of homopolymers as bacteria have evolved a number of mechanisms to decorate and/or heterogenize their biofilm polysaccharides [7,11,26] (Figure 1). For example, the Pel system of *P. aeruginosa* uses the cytosolic PelF GT to polymerize UDP-N-acetylgalactosamine (UDP-GalNAc) into a homopolymer extruded through the PelG translocation pore in the periplasm, where the nascent chain is partially deacetylated by the PelA deacetylase-hydrolase enzyme to yield a positively charged polymer [11,27]. The latter — once funneled through the PelC–PelB OM export tandem — would then cross-link negatively charged eDNA in the stalks of the typical mushroom-shaped biofilm structures [27]. Similarly, alginate-secreting mucoid *P. aeruginosa* polymerizes GDP-D-mannuronate via the bifunctional GT Alg8 that also translocates the polymer across the IM. Once in the periplasm, the polymer is subject to both partial D-mannuronate to L-guluronate epimerization by the periplasmic AlgG subunit and to O-acetylation by the AlgIFJX complex [11] (Figure 1).

Similarly, secreted cellulosic polymers come in a variety of shapes and flavors [7] (Figure 1). Some bacteria, such as members of the *Gluconacetobacter* lineage, feature longitudinal arrays of synthase terminal complexes (TCs) whose secreted, chemically pure cellulose chains bundle into so-called cellulose ribbons that lead to the formation of thick biofilm mats with a high cellulose crystallinity index [28,29]. Many enterobacteria, such as *E. coli*, *S. enterica* serovar Typhimurium, and others, secrete a chemically modified cellulose where up to half of the glycosyl moieties feature pEtN residues at the C^6 position [30]. The pEtN-transferase function is carried out in the periplasm by BcsG, an inner-membrane-embedded, Zn-dependent enzyme from the alkaline phosphatase superfamily that likely partakes in direct but more dynamic interactions with the biosynthetic BcsAB platform [7,30–33]. Indeed, bacterial BcsG was found to interact with an *E. coli*-specific N-terminal domain (NTD) of the BcsA synthase [17] and a separate cryo-EM study of the Bcs macrocomplex attributed tubular densities within the detergent micelle to two copies of $BcsG^{NTD}$, whose poorer resolution and lack of corresponding resolved densities for the C-terminal catalytic domain indicate highly heterogeneous occupancy [33]. Interestingly, whereas in some species and strains BcsG is dispensable for secretion of non-modified cellulose in vivo (e.g. in AR3110, UTI89, or the naturally BcsG-deficient rUT12 [30,34]), in others, it appears required for cellulose biogenesis. For example, in *E. coli* 1094, a nonpolar *bcsG* deletion effectively abolishes cellulose secretion [17]; similarly, in *S. enterica* serovar Typhimurium not only does the transmembrane NTD of BcsG appear to influence the integrity of the cellulose synthase, but also BcsG point mutants carrying a catalytically inactive BcsG C-terminal domain do not secrete cellulose even in the presence of wild-type BcsA levels, thus suggesting direct requirement for the pEtN modification itself [32]. The pEtN-derivatized cellulose has been further shown to determine biofilm architecture and tensile strength by directly interacting with and affecting the polymerization dynamics of the other major enterobacterial matrix component, amyloid curli [35,36]. Finally, some species such as *Pseudomonas fluorescens* SBW25 and *Orrella dioscoreae* feature *wss* gene clusters, whose products are proposed to introduce a different chemical modification — cellulose acetylation — that can affect the biofilm strength, architecture, and/or the overall ecological success of the species [26,37,38]. The predicted Wss subunits share structural and functional homology with the AlgIFJX components from the alginate acetylation complex and include a membrane-

bound O-acyltransferase (MBOATs WssH and AlgI, respectively) proposed to flip acetyl moieties from as-yet uncharacterized cytosolic donors through the IM, one or more immunoglobulin-like adaptor proteins (WssG/AlgF), and SGNH hydrolase-like O-acyltransferases proposed to act on the nascent polymers in the periplasm (WssI/AlgJ and WssF/AlgX) [7,9,11,26,39,40]. Indeed, recent studies on purified WssI homologs from *P. fluorescens* and the multi-organ pathogen *Achromobacter insuavis* have demonstrated in vitro acetylesterase and acetyltransferase activities using a variety of rationally designed acetyl donors and cellooligosaccharidic substrates [39]. Nevertheless, the exact structures, stoichiometry, and functional roles of the putative cellulose acetylation complexes, as well as the exact composition of the modified extracellular polymers, remain to be further experimentally examined.

Periplasmic and outer membrane export

Once exported in the periplasm, the secreted EPS need to cross the peptidoglycan mesh and outer membrane, which is generally achieved through a tandem of a TPR-rich periplasmic scaffold and an outer membrane β-barrel porin [7,11]. The two modules can be part of the same (e.g. BcsC in cellulose, PgaA in PNAG, and PelB in Pel secretion) or different proteins (e.g. AlgK–AlgE in alginate secretion), and the number of TPR motifs and porin β-strands can vary across systems [7,11]. In some cases, export can be further facilitated by additional components such as the PelC lipoprotein, which is proposed to form an OM-proximal dodecameric ring whose negatively charged lumen would funnel the cationic Pel EPS through to the PelB porin domain [41].

The TPR-rich periplasmic scaffolds likely adopt flexible solenoid folds that extend across the periplasmic space [7,11,42] and can recruit additional functional partners. Whereas functional data on BcsC's NTD are currently limited, studies on its functional homolog PgaA from the PNAG secretion system have shown that the protein binds both PNAG and the periplasmic enzyme PgaB to stimulate the latter's deacetylase and glycoside hydrolase activities [43]. Similarly, AlgK recruits the periplasmic O-acetyltransferase AlgX in a stable, alginate-binding complex that is key to periplasmic polymer modification and downstream biofilm attachment [44]. In *E. coli*, BcsC features 19 TPR repeats in its N-terminal periplasmic region and a 16-stranded β-barrel porin domain with a large, electronegative lumen constricted near the extracellular surface and lined with conserved polar and aromatic residues. An ~15-residue-long proline-rich C-terminal extension folds into the channel to position the last aromatic (W^{1157}) residue midway across the lumen [45]. These features, conserved across pEtN-cellulose-secreting bacteria, likely prevent permeability for solutes in the resting state while facilitating the take-up and outward gliding of hydrated, zwitterionic pEtN-cellulose [7,45].

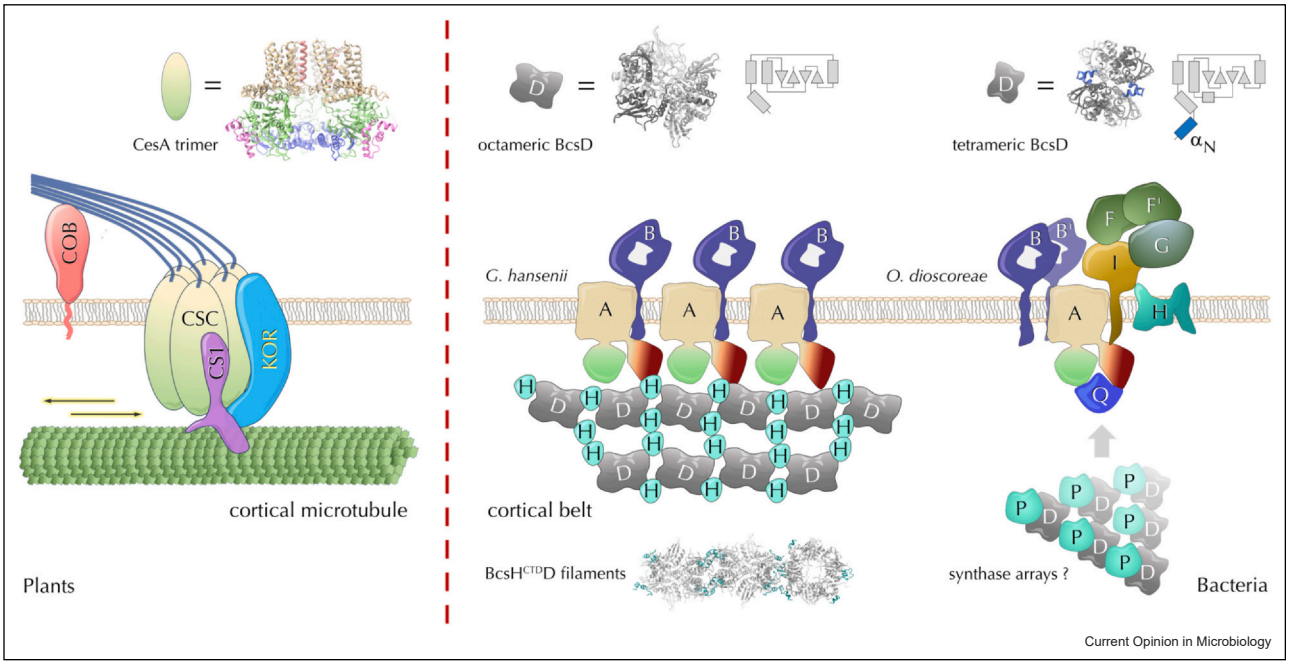
Interestingly, many *bcs* clusters do not feature a *bcsC* gene but instead encode BcsK, which has multiple TPR motifs but no obvious porin domain, so how the polymer crosses the outer membrane has remained enigmatic [7,10] (Figure 1). Finally, some cyanobacterial systems likely feature a very different, Type-I protein secretion system-like Bcs architecture where the BcsB copolymerase and BcsC are substituted by proteins similar to the membrane fusion protein HlyD and the outer membrane efflux protein TolC, respectively [46].

Intracellular cytoskeletal scaffolds

Crystalline cellulose secretion by bacteria from the *Gluconacetobacter* lineage has long attracted interest as a source of chemically pure polymer for a variety of biotechnological applications [47]. Almost half a century ago, freeze-fracture and negative-stain electron microscopy experiments visualized the longitudinal linear assembly of synthase TCs, their colocalization with the exit points of secreted cellulose microfibrils, and the latter's extracellular bundling into a single crystalline cellulose ribbon per cell [28]. Two different Bcs proteins were subsequently identified as cellulose crystallinity factors: BcsD (CesD) and BcsH (CcpAx). BcsD is a small, ~17-kDa protein, which in *G. hansenii* assembles into donut-shaped D4-symmetric octamers featuring 4 independent luminal passages capable to bind cellulosic oligosaccharides [48,49]. This peculiar architecture had led to a functional model in which BcsD serves as a periplasmic guide for individual cellulose chains exiting the BcsA translocation pores and fluorescence-based imaging studies showed that indeed BcsD localizes in a longitudinal line along the cell body, similarly to the synthase TCs [48,50]. Importantly, this linear BcsD localization is required for crystalline cellulose biogenesis and depends on direct interactions with the second crystallinity factor BcsH, initially proposed to be a short, ~8-kDa periplasmic peptide [50].

Recently, an in situ cryoelectron tomography study of *Gluconacetobacter* cells and biofilms revealed a cytoskeletal structure, dubbed the 'cortical belt' [29] (Figure 3). The cytosolic assembly, tens of nanometers wide and hundreds of nanometers long, was shown to run in one to several stacked sheets parallel to the extracellular cellulose ribbon and at a fixed distance (~24 nm) from the IM [29]. In addition, cryo-EM and functional studies on the BcsHD tandem showed that BcsH is in fact an ~37-kDa protein that assembles the BcsD octamers into 'beads-on-a-string' filaments via its short C-terminal domain (Figure 3), whereas the N-terminal proline-rich region is capable of self-oligomerization, likely driving the BcsD filaments into three-dimensional sheets and

Figure 3



Cytoskeletal scaffolds for cellulose secretion. Left, cortical microtubules and plant cellulose synthase complexes (CSCs or rosettes); CSI, cellulose synthase-interacting protein 1; KOR, KORRIGAN β-1,4-endoglucanases; COB, COBRA cellulose-binding proteins in the apoplast, membrane-anchored; CesA, a eukaryotic cellulose synthase.
(adapted from reference [52]). Right, bacterial cytoskeletal scaffolds of octameric or tetrameric BcsD and proline-rich partners. Structural data from references [29,37,48,49,51].

stacks [49]. Importantly, bacterial two-hybrid (BACTH) functional complementation assays revealed interactions of the crystallinity factors with the cytosolic BcsA^{PilZ} domain, whereas in situ cryo-EM visualization at higher resolution revealed striking similarity between the cortical belt profile and the BcsHD 'beads-on-a-string' filaments [49]. These findings, together with the lack of secretion signals on either BcsD or BcsH, support an updated model of inside-out regulation, where the two proteins assemble into the intracellular cortical belt to drive longitudinal TC array formation and crystalline cellulose biogenesis [49] (Figure 3).

Whether BcsD and BcsH use an as-yet uncharacterized export process to provide additional roles as polysaccharide conduits in the periplasm remains to be further investigated. The inside-out hypothesis was recently further enforced by structure–function studies on BcsD homologs from diverse bacteria, including species expected to secrete acetylated (*O. dioscoreae*) or pEtN-modified (*Enterobacter* sp. 638) cellulose [37]. Cryo-EM structures of BcsD from several β- and γ-Proteobacteria revealed that through a conserved additional N-terminal helix, BcsD can switch from D4-symmetric octamers to D2-symmetric tetramers [37] (Figure 3). The latter in turn can interact with previously uncharacterized proline-rich partners (BcsP in β- and BcsO in γ-Proteobacteria), as well as with the synthase-binding ATPase BcsQ, to form intracellular supramolecular scaffolds that determine the efficiency of cellulose secretion and the overall biofilm strength and architecture [37]. Unlike the BcsHD filaments that drive the assembly of longitudinal TC arrays and a crystalline cellulose ribbon in *Gluconacetobacter*, the BcsD–BcsP complexes from *O. dioscoreae* were shown to array into triangular tiling modules likely determined by the tetrahedron-like architecture of the individual BcsD tetramers [37] (Figure 3).

How in each case these atypical cytoskeletal scaffolds assemble and whether they serve to recruit additional regulatory components remains to be further examined. Nevertheless, enlisting cytoskeletal elements into the regulation and spatial organization of cellulose synthase activity appears to have evolved multiple times in evolution. Indeed, in plants, the mature cellulose synthase complexes (CSCs) are delivered from the *trans*-Golgi network to the cell membrane, where they are linked to cortical microtubules via CSI1 [51,52], among others, and activated by phosphorylation (Figure 3). Whereas in plants the ensuing synthesis of crystalline cellulose is proposed to generate forces propelling the CSC rosettes within the membrane and along the cortical cytoskeleton in a process required for cell wall biogenesis [52], in

Gluconacetobacter similar forces and cortical belt-anchored, static TCs are believed to convert the secreted cellulose ribbon in a *bona fide* motility organelle during substrate colonization [28,29].

Conclusions and outlook

Secreted cellulosic polymers represent key architectural and functional constituents of the three-dimensional biofilms of many free-living and host-associated bacteria and are produced in both temporally and spatially regulated manner by dedicated multicomponent Bcs secretion systems [7]. Although these demonstrate functional similarities with other synthase-dependent EPS-secretory assemblies and even evolutionary distant eukaryotic systems for cellulose biogenesis, they also present important mechanistic idiosyncrasies that have only recently begun to emerge. Although bacterial cellulose biogenesis represents the longest-studied process for synthase- and c-di-GMP-dependent EPS secretion [53], it remains to be fully understood how subsets of biofilm cells switch to an energetically expensive, sugar-consuming anabolic process in typically nutrient-limited conditions to provide the scaffold and ramparts of the mature macrocolony; how in each system activating c-di-GMP is relayed to and recycled by the cellulose synthase; how the latter interacts with polymer-modifying enzymes that heterogenize the nascent chains for novel functionalities, and/or with cytoskeletal elements to form higher-order biosynthetic arrays for increased polymer crystallinity; how these flexible polysaccharides are guided through the periplasmic peptidoglycan mesh and to the cell surface; or how they interact with additional extracellular matrix components from the parent bacteria, synergistic or competing community microbes, or colonized eukaryotic hosts. Advancing our current understanding of cellulose biogenesis and modifications could allow us not only to design strategies for biofilm prevention and dispersal, but also to engineer cellulose superproducers and/or chemi-enzymatic systems with enhanced probiotic, biocontrol, or industrially relevant properties.

CRediT authorship contribution statement

Petya V. Krasteva: Conceptualization, Data curation, Visualization, Funding acquisition, Writing – original draft, Writing – review & editing.

Data Availability

No data were used for the research described in the article.

Declaration of Competing Interest

The author declares that she has no known competing financial interests or personal relationships that could have appeared to influence the work reported in this paper.

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Data availability

No new or unpublished data were reported in this work.

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- • of special interest
- •• of outstanding interest

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