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Surface Sensing and Adaptation in Bacteria

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Abstract

Bacteria thrive both in liquids and attached to surfaces. The concentration of bacteria on surfaces is generally much higher than in the surrounding environment, offering bacteria ample opportunity for mutualistic, symbiotic, and pathogenic interactions. To efficiently populate surfaces, they have evolved mechanisms to sense mechanical or chemical cues upon contact with solid substrata. This is of particular importance for pathogens that interact with host tissue surfaces. In this review we discuss how bacteria are able to sense surfaces and how they use this information to adapt their physiology and behavior to this new environment. We first survey mechanosensing and chemosensing mechanisms and outline how specific macromolecular structures can inform bacteria about surfaces. We then discuss how mechanical cues are converted to biochemical signals to activate specific cellular processes in a defined chronological order and describe the role of two key second messengers, c-di-GMP and cAMP, in this process.

1. INTRODUCTION

In natural ecosystems, bacteria can exist as free-swimming, individual cells navigating their environment with the objective of locating favorable niches. Alternatively, bacteria thrive as sessile communities populating abiotic and biotic surfaces. The ability to associate with surfaces has played an important role in the successful colonization of nearly every terrestrial environment sampled to date. Likewise, it is estimated that even in the largest aquatic environments, the oceans, with their vast reservoir of microbial life (216), microbes are concentrated in floating microparticles originating from dead plankton and other organic matter

(187). Compared to the nutrient-depleted aqueous environment, organic particles and other nutrient-rich surfaces provide substantial nutritional benefits and may thus represent the foremost habitats of bacteria on earth (187). The concentration of bacteria on surfaces (including animals and plants) is orders of magnitudes higher than in the surrounding environment (55), offering bacteria ample opportunity for mutualistic, symbiotic, and pathogenic interactions. To successfully populate surfaces, bacteria need to be able to sense physicochemical stimuli upon approaching a solid substratum and respond to these cues by adapting their physiology and behavior accordingly. For instance, bacteria can change their motility behavior to explore surfaces, prepare for their interaction with host tissue and defense systems, and produce specific adhesins to adhere and eventually irreversibly attach to surfaces.

Surface attachment and colonization are initial steps of the formation of biofilms, structured surface-bound bacterial communities that grant a series of survival advantages. These advantages include protection from predators, flow shear forces, and other adverse effects of the environment; safeguarding from the host's immune defenses (171); increased tolerance to antibiotics (74, 145); enhanced opportunities to share genetic information; and the ability to cooperate and to potentiate their activities via the expression of toxins and exoenzymes (105, 132). While some of these traits emerge as soon as bacteria contact surfaces (154, 165), other properties develop only later during surface-based growth and biofilm maturation. Given the relevance of surface-grown microbial communities in the environment (97), medicine (173), and industry (157), a detailed understanding of the processes leading to surface colonization is imperative and will help in designing strategies to effectively counter unwanted bacterial interactions.

To adhere to surfaces, bacteria need to be in physical contact with a substratum, host cells, or neighboring bacteria. Although bacteria can adhere to surfaces passively, flagellar motility increases their chance to encounter a surface (201) (**Figure 1**). Bacteria swimming close to a surface can use pili to make contact with the solid substratum, thereby adopting circular orbits and increasing the time spent in close proximity to the surface (39, 48, 118). Surface-released nutrients serve as chemoattractants, e.g., in the case of marine particles and plant roots (52, 187). In addition, flagella can serve as adhesins and increase the chances to remain on the surface (22, 56). Other cellular appendages, in particular pili, contribute to successful attachment and can act as fishing rods to contact surfaces (13). Ultimately, bacterial adhesion is determined by a balance

between Brownian motion, repulsive electrostatic forces, Lifshitz–Van der Waals forces, and hydrogen bonding, forces that vary widely depending on the chemical and physical properties of different surfaces (143) (**Figure 1**). This review does not discuss physical and chemical parameters of bacterial surface adhesion in detail. These aspects are covered extensively in some excellent recent reviews (26, 201).

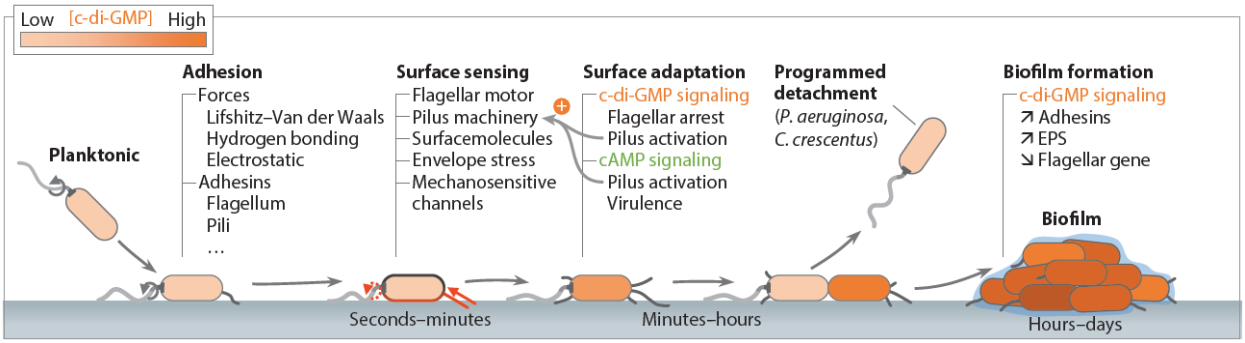

Figure 1 Timeline of bacterial surface adaptation leading to biofilm formation.

Bacteria can use chemical (129, 158) and mechanical (29, 69) sensing to respond to surfaces. Numerous examples exist for bacterial surface adaptation, from specialized adhesin or changes in motility behavior taking place on a timescale of seconds or minutes (87, 189) to processes relating to surface growth and biofilm formation on a timescale of hours or days (84) (**Figure 1**). Because these processes are often difficult to access and clearly distinguish experimentally (92), the exact molecular mechanisms mediating surface responses often remain ill defined. This review provides an overview of the current models and proposed mechanisms of the sense of touch used by bacteria, a phenomenon that is also referred to as mechanosensation and mechanotransduction. Several excellent recent reviews cover this topic from different angles (29, 92, 153, 155). However, some aspects need clarification. First, the literature often does not make a clear distinction between the terms mechanosensation and mechanotransduction or between surface sensing and surface adaptation. While the former refers to the mechanisms responsible to convert a mechanical signal into a (bio)chemical output, the latter describe downstream processes leading to specific cellular responses. Finally, because bacterial surface colonization is a gradual development with successive processes occurring at different timescales, timing aspects need to be defined precisely. Here, we attempt to be as specific as possible when commenting on the respective timescales of individual surface processes.

2. SURFACE MECHANOSENSING

2.1. Mechanosensing Through the Flagellar Motor

Swimming bacteria use a molecular motor to drive the rotation of a long helical filament, the flagellum (12, 134, 139, 185). The flagellar motor is embedded in the membrane and cell wall and is powered by a gradient of protons (H^+) or sodium ions (Na^+) (**Figure 2*a***). The force generators of the motor, the stator units, are multimers composed of MotA and MotB. Below, we discuss the important role of the flagellum as a mechanical surface-sensing device.

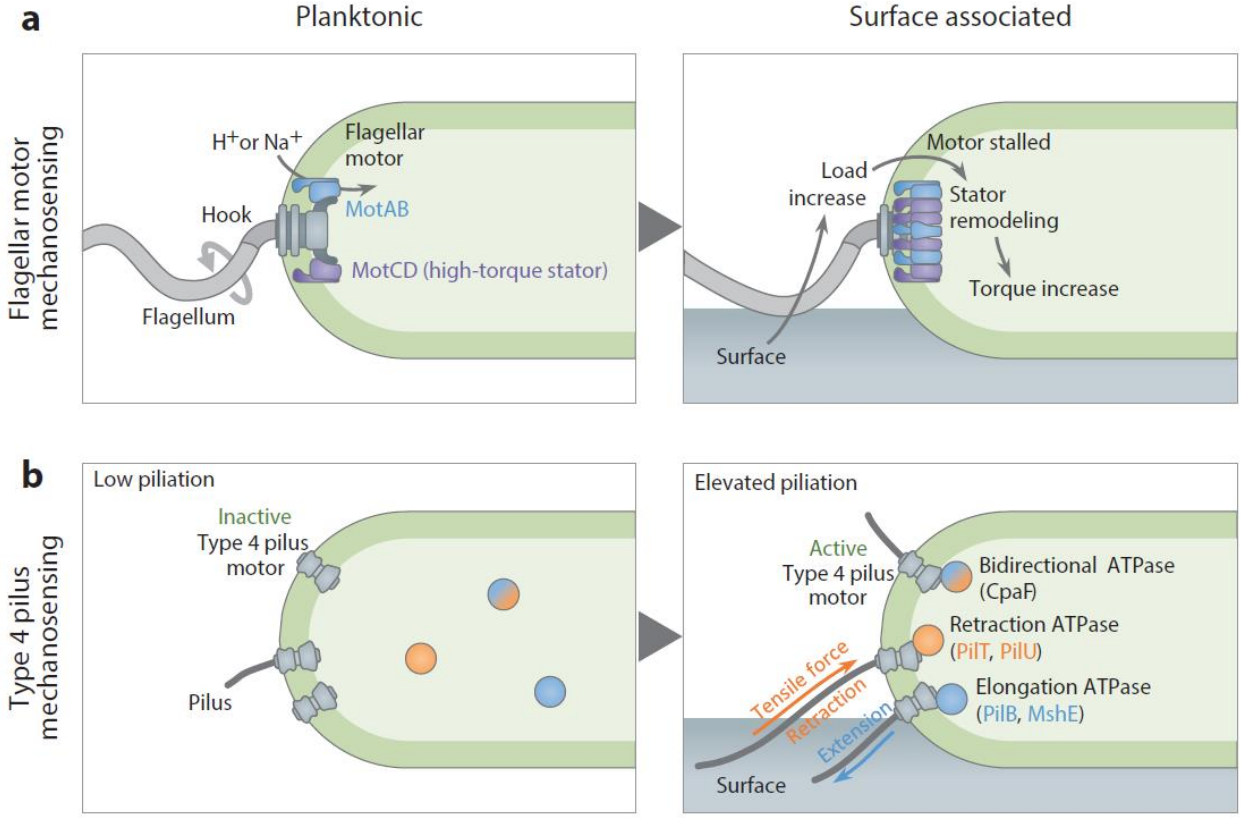

Figure 2 Surface mechanosensing by flagellar and type IV pilus (T4P) machineries. (*a*) Flagellar rotation is driven by a flux of H^+ or Na^+ through the membrane-embedded stator (MotAB). Surface contact increases the load on the flagellum. At short timescales, motor stalling leads to stator remodeling by recruiting new stator units or by exchanging existing stator sets with stators that can generate higher torque (e.g., MotCD in *Pseudomonas aeruginosa*). Besides sensing motor stalling or remodeling, the signal could be transduced by sensing envelope deformation, local pH changes, or transient changes of the membrane potential. (*b*) Adhesive T4Ps tether the cell body to the surface. Active retraction of T4Ps by ATPases (PilT, PilU, CpaF) generates a tensile force, which could be sensed as an increase of motor load or by detecting stretched pilus conformations.

2.1.1. Flagellar motility promotes surface colonization.

Early observations had indicated that the flagellum and flagellum-based motility contribute to the successful colonization of surfaces. *Escherichia coli* mutants with a paralyzed flagellum, but not

chemotaxis mutants, showed strong defects in biofilm formation (161). Likewise, a mutant of *Pseudomonas aeruginosa* lacking a complete flagellar structure showed poor attachment to plastic surfaces, even after extended incubation (145b). Flagellar and motor mutants of *Vibrio cholerae* also showed poor surface attachment (205). The polar flagellum of *V. cholerae* is powered by a sodium gradient (ΔpNa) generated by the Na^+ NQR pump. Interestingly, *nqr* mutations affecting the sodium motive force and flagellar motor performance disable *Vibrio*'s capacity to transit from transient to permanent surface attachment (205). Similarly, interference with the function of the polar flagellum blocks the formation of surface-adapted *Vibrio parahaemolyticus* swarmers (11, 89, 122). These observations were attributed to two possible functions of flagella: (*a*) active movement toward surfaces to increase the probability of surface encounter, and (*b*) activity of the flagellum as a powerful adhesin, promoting stable interaction of bacteria with surfaces (57). Moreover, swimming bacteria experience a basal drag force that stops once cell bodies are tethered on a surface, possibly interpreted by the cell as a landing event (29).

2.1.2. The role of the flagellar motor in surface sensing.

Work by McCarter and colleagues (122) had introduced the idea of the flagellar machinery acting as a dynamometer. They observed that in *V. parahaemolyticus* a decrease in rotation speed triggers the expression of lateral flagella for swarming. Together with other observations, this led to the idea that upon surface encounter, an increased load on the stator would inform cells on the new conditions (10, 25, 28, 122, 199). But what would be the responsible molecular mechanism(s)? Increasing evidence argues that torque-dependent remodeling of the flagellar motor could be exploited by bacteria to sense their physical environment. Although a single stator unit can achieve maximum swimming speed in near-zero-load conditions (185), conditions that mimic surfaces by increasing the flagellar load (increased medium viscosity, attachment of beads to the flagellum) were shown to trigger the recruitment of additional stator units (10, 109, 140, 197) (**Figure 2*a***). In *E. coli*, high loads and torque enhance the interaction of the stator units with the rotor, while paralyzed stators only weakly interacted with the rotor (28, 208). Lele and collaborators (109) observed an instant decrease of the rotational speed from 300 to 10 Hz upon attachment of latex beads to individual flagella. However, within the first 10 s, rotation speed gradually recovered to reach a new plateau after about 2 min, a process that coincided with the recruitment of additional stator units. These observations indicated that stator units are

mechanosensitive, with the number of engaged units depending on the viscous load on the motor (**Figure 2*a***). The observation that the lifetime of an assembled stator unit increased at higher force implied that mechanosensation by the *E. coli* flagellar motor complex is driven by a catch bond mechanism. These are noncovalent bonds that increase in strength with increasing tensile force, meaning that in this context the binding of each stator unit is enhanced by the force it develops (28, 140). In *P. aeruginosa* MotAB is required for rapid surface sensing (104, 179), and the polar positioning of the flagellum is important for proper surface response (179). In *Bacillus subtilis* load-dependent motor remodeling was also observed for the Na^+-driven stator MotPS at increased viscosity (194). Flagellum-based surface sensing appears to be a common mechanism in bacteria.

2.1.3. The tetherless model of flagellar surface sensing.

Although in the initial experiments the load was applied to the flagellar filament, load can also be exerted on more proximal parts of the flagellum. Recent reports indicated that the flagellum is able to sense surfaces even when it lacks some of its external parts and that this response may primarily rely on the membrane-embedded motor components (109). For example, in *Caulobacter crescentus* flagellum-mediated surface sensing leads to the production of the second messenger c-di-GMP and synthesis of a polar adhesin, the holdfast (79, 110). Although surface sensing clearly depends on active stator units, external flagellar components were not required for this response (79). Similarly, upon encountering a surface, *P. aeruginosa* cells experience an increase in their c-di-GMP concentration within seconds, a process that depends on the MotAB stator but does not require the flagellar filament FliC (104).

2.1.4. Molecular mechanisms for flagellum-mediated surface sensing.

A number of possible mechanisms were proposed to explain the role of flagellar motors in surface sensing. (*a*) Dynamic changes of stator components could be sensed either by recording the changing number of free stators (stator depletion) or by directly monitoring the stoichiometry of the motor. A stator depletion mechanism seems unlikely, as bacteria with a single or few flagella per cell would require highly accurate counting devices to detect a few free stators missing in a pool of hundreds of free stators (29). (*b*) The increase of torque could deform the cell envelope, thereby activating mechanosensitive membrane proteins (9; see Section 3). In principle, the motor-associated ion-conducting stator proteins themselves could serve such a

function. (*c*) Bacteria could sense the local membrane potential or local pH changes as proton or sodium flux changes as a result of motor stalling or remodeling (25, 29, 79, 205). Physically associated sensors of motor performance were proposed in *C. crescentus* (79) and in *B. subtilis*, where the diguanylate cyclase DgcB and the two-component system DegSU are activated, respectively, when the flagellar machinery is compromised (25). Finally, the role of the flagellum as a mechanosensitive device may rely on its coordinated action with other organelles like adhesive pili. For example, it was recently proposed that in *C. crescentus* retracting type IV pili (T4Ps) mediate the positioning of the flagellated pole close to the surface, thereby facilitating motor-mediated mechanical sensing (176). The authors proposed a model in which flagellum and pili functionally interact and together impose a ratchet-like mechanism that progressively drives *C. crescentus* cells toward permanent surface attachment.

2.2. Mechanosensing by Surface-Exposed Pili

Bacteria have evolved different types of adhesive pili or fimbriae to adhere to surfaces. Some of these pili appear to have adopted roles in mechanical sensing. For instance, type I pili of uropathogenic *E. coli* (UPEC) mediate attachment to host tissue via their sugar-binding adhesin FimH (76). Displayed at the pilus tip, FimH mediates adhesion to mannosylated host epithelial cells via its lectin domain (33). A sophisticated mechanism allows UPEC to balance tight attachment to host tissue with their dissemination through the urinary tract. In response to shear forces caused by urine flow, FimH can transition from a low-affinity to a high-affinity binding mode. This catch-bond mechanism thus optimizes cell-cell adhesion under dynamically varying conditions and mechanical stress (177, 184, 195).

While type I pili are largely static entities undergoing conformational change in response to shear forces in their environment, T4Ps are much more sophisticated, highly dynamic, and multifunctional molecular tools found in most gram-negative pathogens (44, 149). T4Ps are long extracellular polymers of the PilA protein that can elongate and retract. The pili machinery is embedded in the cell envelope with motor ATPases driving PilA polymerization and depolymerization being located in the inner membrane (27) (**Figure 2*b***). T4Ps ensure stable surface attachment and promote early stages of biofilm formation (31, 73, 93, 119, 145b, 218). As T4Ps can attach to surfaces, specifically or nonspecifically, their retraction can pull bacteria forward in a surface locomotion named twitching (23). But the role of T4Ps is not limited to their adhesive properties or surface motility. Recent evidence points to a specific role for T4Ps in

surface sensing and in initiating surface signaling cascades. Similar to the case of the flagellum, the load on the pilus motor increases when cells are tethered to the surface or when they engage in cell-cell contact. While no tensile forces are applied to pili in liquid medium, surface-attached pili can generate forces from 8 to 100 pN (37, 50, 117, 126, 176). To generate tensile force, cells must be in contact with a surface via tethered pili, and pili must actively elongate and retract. Thus, pili-based mechanosensing requires surface-attached dynamic pili.

2.2.1. Molecular mechanisms of T4P-mediated surface sensing.

Because most studies on the role of T4Ps in surface sensing use indirect assays, the exact molecular details remain poorly defined (153). Several models have been put forward, including the obstruction model and the stretched pili model. Similar to the flagellar load model, the former proposes that pili measure increased load during pilus retraction. This model is supported by the observation that obstructing pilus retraction in *C. crescentus* stimulates the production of a polar adhesin, the holdfast, a process known to respond to surface encounter (50). Similarly, retraction of tethered T4Ps in *P. aeruginosa* caused an increase in cAMP within 30 min after surface contact, a response that depends on intact motor ATPases (154). Monitoring pili dynamics by interferometric scattering microscopy revealed that motors of tethered pili specifically recruit the retraction ATPase PilT (193) (**Figure 2*b***). Remodeling of the pilus motor under high-tension regimes is reminiscent of flagellar motor remodeling at high-torque regimes described above. An alternative model proposed that the force experienced by tethered retracting pili leads to a conformational change of the pilus filament (96, 198) and that bacteria can exploit this information to gauge surface encounter. In *Neisseria gonorrhoeae*, stretched pili can extend up to threefold in length as compared to their relaxed conformation, thereby exposing new epitopes that were previously buried in the filament (17). Similarly, the major pilin subunit PilA of *P. aeruginosa* was shown to adopt a specific stretched conformation under tension (8). Recognition of specific pilin epitopes or conformations could in theory inform bacteria about their physical environment. This idea is supported by the finding that in *P. aeruginosa* free PilA subunits (i.e., not assembled in a pilus filament) directly interact with the chemoreceptor-like protein PilJ, which itself drives the surface-induced increase of cAMP (154). This observation led the authors of this study to propose that PilJ contributes to surface sensing by monitoring conformational changes of PilA.

2.2.2. PilY1, a putative mechanosensor associated with T4Ps.

P. aeruginosa possess another putative surface-exposed mechanosensor, the outer membrane protein PilY1 (115, 183). Because the *pilY1* gene is part of the T4P gene cluster and upregulated when *P. aeruginosa* is grown on a surface, and because its secretion depends on the pilus machinery, it was speculated that PilY1 is involved in a mechanosensory pathway that is coordinated with the T4Ps (115). PilY1 harbors a domain with homology to Domain A of the mechanosensitive von Willebrand factor. Consistent with a role as mechanosensor, PilY1 activates the production of the second messenger c-di-GMP in a surface-dependent manner and only when it is secreted to the cell surface (100, 183). Deletion of the von Willebrand factor A domain of PilY1 caused constitutive expression of surface-specific virulence genes even in the absence of a surface (183). PilY1 was also proposed to play a role as adhesin (70, 85) or as an antiretraction factor of T4P (138), adding yet another level of T4P-mediated surface response.

2.3. Flagella and Pili: Successive or Coordinated Action?

Above we summarized experimental evidence in support of flagella and pili playing important roles in surface sensing. It is less clear whether these surface organelles operate independently or whether their contribution to surface recognition is coordinated. In the latter case, polar colocalization of the flagellar machinery and T4Ps may be essential for the coordination of the surface response (176) and is ensured by hub proteins (e.g., FimV in *P. aeruginosa*) (80, 181, 215).

Flagellar motor–dependent surface sensing in *P. aeruginosa* was shown to be rapid (<20 s) and to be independent of the presence of T4Ps (104). In comparison, T4P-dependent surface response appears to take place on a longer timescale (<1 h) (154). However, while the assay of the first study makes use of a posttranslational reporter system, the second study relied on cAMP-dependent transcriptional reporters entailing protein synthesis and folding and thus possibly underestimating the speed of T4P-mediated surface sensing. Alternatively, flagellum- and T4P-mediated surface sensing ensue in succession and on different timescales, with the former being important during the initial events of surface encounter (seconds) and the latter consolidating subsequent processes of surface adaptation (minutes to hours). In line with this is the observation that planktonic *P. aeruginosa* cells generally lack pili but are rapidly piliated when encountering a surface, a process that depends on the flagellar motor and on downstream signaling events (42, 104, 154).

The situation is different in *C. crescentus*. Motile swarmer cells carry a flagellum and pili at the same pole, arguing that the two organelles likely cooperate to promote surface attachment. However, recent reports are contradicting in that Hug et al. (79) found that the flagellar motor but not pili are required for surface sensing, while Ellison et al. (50) showed that in their experimental setup pili mediate a surface program independent of the flagellar motor. It is possible that these discrepancies relate to distinct experimental conditions used in these studies and that the sensory functions of flagella and pili are highly specialized. Also, while both studies monitored holdfast formation as a direct readout for surface, holdfast production is either monitored in real time in microfluidic devices or in batch cultures after prolonged washing steps. Thus, it is possible that the two experimental setups record similar processes but on different time axes. In contrast to these observations, a recent model proposed that a flagellum and pili functionally interact to progressively drive *C. crescentus* cells toward permanent surface attachment (176).

3. PHYSICOCHEMICAL SIGNALS USED FOR SURFACE SENSING

Apart from being able to sense surfaces through specialized cell appendages and motors, bacteria can monitor surface-induced envelope stress and deformation, local pH changes, or specific chemicals that are released by surfaces. While most of these signals are not surface specific, bacteria may have evolved ways that allow them to interpret some of these cues as proxies for surface sensing.

3.1. Recognition of Surface-Specific Chemistry

To recognize host tissue and induce a surface-specific virulence response, an alternative way to probe the mechanical environment is to sense molecules that are specific for the host surface. For example, *V. cholerae* is able to adhere to the chitin exoskeleton of its natural arthropod hosts via specific chitin-binding proteins and type IV mannose-sensitive hemagglutinin (MSHA) pili (125, 163). Secretion of chitinases releases *N*-acetylglucosamine, the building block of chitin, from the surface, which in turn is sensed by the membrane-bound histidine kinase ChiS (111). Thus, although sensing *N*-acetylglucosamine does not require a surface per se, its presence manifests host proximity and triggers surface adaption and competence by promoting the expression of type IV ChiRP pili and by inducing biofilm formation (3, 125) (**Figure 3*a***). Similarly,

Staphylococcus aureus cells are decorated with fibronectin-binding protein (FnBP) to facilitate adhesion to host tissue and biofilm formation (61). Surprisingly, FnBP molecules were shown to relocalize to regions of the bacterial cell surface that specifically contact fibronectin-coated surfaces (114) (**Figure 3*a***). Thus, chemosensing constitutes an efficient alternative to mechanosensing in cases where the surface habitat has a defined chemistry.

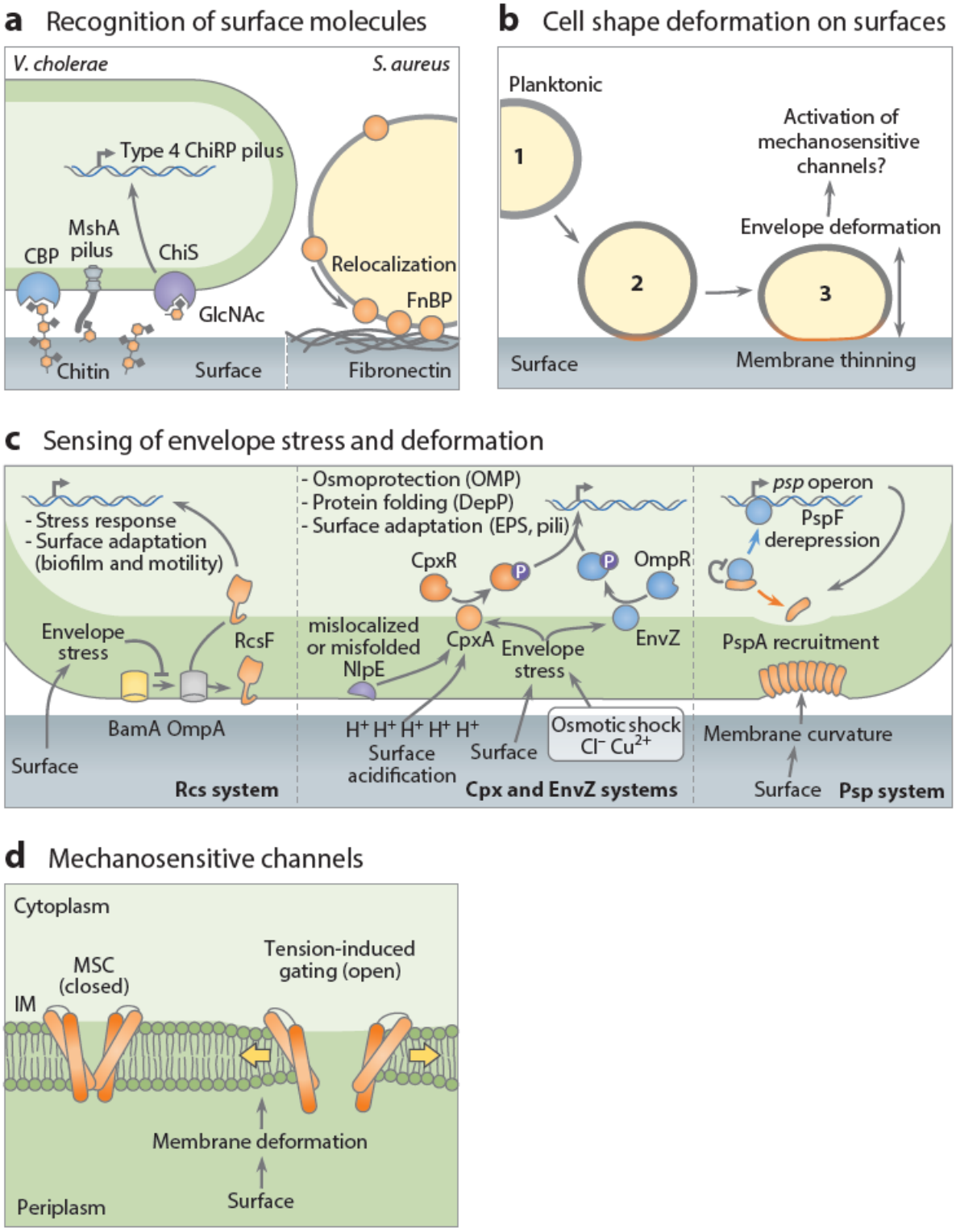

Figure 3 Bacterial surface sensing through the recognition of surface molecules and envelope stress. (*a*) *Vibrio cholerae* recognizes chitin surfaces using specific chitin receptors (CBP and MSHA pili) or its degradation product GlcNAc (histidine kinase ChiS). *Staphylococcus aureus* specifically binds surface host cell surfaces using the fibronectin receptor FnBP, which is able to relocalize to points of surface contact. (*b*) Adhesion to surfaces leads to envelope deformation (up to 10% of the diameter of the bacterium) and to membrane thinning at the point of surface

contact. (*c*) Overview of the main envelope stress detection systems. The Rcs system detects misassembly of outer membrane proteins. When RcsF is not properly translocated, it promotes the transcription of stress response and surface adaptation genes. The Cpx and EnvZ/OmpR systems integrate several signals, including mechanically inflicted envelope stress or surface acidification (0.5–2 pH units) in the first hours of surface exposure. Both systems trigger a transcriptional adaptation to cope with osmotic stress, rescue misfolded proteins, and eventually adapt to the surface environment. The Psp system senses altered membrane curvature by recruitment of PspA to prevent membrane rupture. (*d*) Contact with a surface can deform the membrane and lead to the opening (tension-induced gating) of mechanosensitive channels. Abbreviations: CBP, chitin-binding protein; EPS, exopolysaccharide; FnBP, fibronectin-binding protein; GlcNAc, *N*-acetylglucosamine; IM, inner membrane; MSC, mechanosensitive channel; MSHA, mannose-sensitive hemagglutinin.

3.2. Envelope Stress and Deformation

When bacteria adhere to solid surfaces, the cell is generally deformed by attractive forces of the surface and by tensile forces of retracting pili (30) (reviewed in 26). Electron micrographs of *S. aureus* showed cell envelope deformation in the range of 3–10% of the cell diameter and membrane thinning at the contact point with the surface (63) (**Figure 3*b***). Nanoscopic membrane deformations can be sensed directly by mechanosensitive proteins (26). Moreover, stress imposed by membrane or cell wall deformation (24, 69, 146) can lead to the accumulation of lipopolysaccharide (LPS) intermediates or unassembled outer membrane proteins in the periplasm (47, 112), which are sensed by the general bacterial stress response. In the following, we summarize the contribution of envelope stress and deformation sensing mechanisms to surface adaptation.

3.2.1. Cell envelope deformation by shear forces.

Bacteria experience shear forces on surfaces when exposed to a turbulent flow or when the cell body is dragged by retractile pili. Shear stress promotes surface adhesion and aggregation of wastewater communities (178). Increasing the shear forces applied to surface-attached *P. aeruginosa* cells by increasing the flow rate led to increased c-di-GMP production (169), arguing that shear stress triggers surface adaptation. This process was shown to be dependent on functional T4Ps and PilY1, by a yet unknown molecular mechanism. However, shear forces experienced by bacteria in flow cells are small (<0.01 pN) compared to the viscous drag experienced by swimming cells (1 pN) and the tensile load exerted by T4Ps (100 pN). Thus, the contribution and mechanism of flow-induced shear forces to surface adaptation remain unclear (29). The idea that *P. aeruginosa* can sense flow speed in a force-independent manner (rheosensing) (175) further complicates the interpretation of the effect of shear forces in the context of surface sensing.

3.2.2. Monitoring envelope stress.

Gammaproteobacteria have evolved a system to monitor envelope stress, the Rcs phosphorelay (32). The Bam machinery with its central component BamA assembles outer membrane β-barrel proteins (OMPs) (98). Normally, RcsF is also inserted into the outer membrane via BamA, where it is exposed to the cell surface by OMPs. What exactly RcsF responds to is unclear. One model suggests that RcsF monitors BamA activity (32). An alternative model proposes that RcsF/OMP complexes monitor lateral interactions between LPS molecules (98a). In both models, RcsF activates a complex phosphorelay that induces a genetic program to cope with envelope stress and to adapt to growth on surfaces (209). RcsF is highly versatile and responds to different sources of envelope damage (outer membrane, LPS, or peptidoglycan damage) and surface contact (91). For example, the Rcs phosphorelay was shown to control flagellar motility and surface swarming in *Proteus mirabilis* (131). Likewise, Rcs promotes biofilm formation, but this activity depends on a complex equilibrium of multiple pathways that is not well understood (53, 127, 209).

3.2.3. Monitoring high osmolarity.

The bacterial cytoplasm contains a high concentration of macromolecules and osmolytes resulting in substantial osmotic pressure differences across the membrane. Cells attaching to substrates may experience rapid changes in osmolality in close proximity to surfaces, leading to cell deformation, membrane tension, and possibly changes of the membrane potential (159). The best-characterized osmolarity-sensing system is the CpxAR two-component system, found in *Gammaproteobacteria* and *Firmicutes* (207). Although not a professional surface sensor, CpxAR is able to detect stress induced by the contact of bacteria with a solid substratum (166). The membrane-bound sensor kinase CpxA receives input from several cellular and environmental cues, including misfolded or mislocalized outer membrane lipoprotein NplE, misfolded pilins (207), alkaline pH (45, 120), and the accumulation of chloride and copper ions (207) (**Figure 3*c***). The implications of some of these pathways for surface sensing have remained controversial (46, 91, 146). CpxA-mediated phosphorylation of the response regulator CpxR activates the transcription of a set of genes involved in osmoprotection, envelope stress response (207), and surface adaptation, such as T4P (137) and type I pilus (180) genes, as well as polysaccharide genes for biofilm formation (116). A second osmolarity sensor is the membrane-embedded histidine kinase EnvZ in *E. coli*, which is stabilized by high osmolarity. Phosphorylation of the

cognate response regulator OmpR upregulates the expression of outer membrane proteins (OmpF, OmpC) to regulate the osmotic pressure (212).

3.2.4. Sensing altered membrane curvature.

The phage shock protein Psp allows many gram-positive and gram-negative bacteria to respond to membrane stress. While Psp was originally observed to protect membrane integrity during filamentous phage f1 infection (21), it was later shown to respond to other cues affecting the membrane integrity, including contact-dependent growth inhibition, chemical membrane stresses, alkaline pH shock, biofilm formation, and cell aging (86). Psp was also implicated in sensing membrane curvature stress, a possible consequence of bacteria interacting with stiff surfaces. Membrane curvature, a prestage of membrane rupture, generates hydrophobic microdomains, which recruit PspA to the membrane (123) and lead to the derepression of the transcriptional activator PspF. This results in a specific boost of PspA expression to further stabilize the membrane and prevent cell ruptures (86).

3.2.5. Sensing membrane deformation through mechanosensitive ion channels.

Membrane tension can be sensed by means of membrane-integral mechanosensitive channels (MSCs) found in all living organisms (reviewed in 191). MSCs are physically stretched when the membrane is deformed, thereby modulating their permeability, a process termed tension-induced gating. As discussed above, surface adhesion leads to the deformation of the cell wall, thereby stretching and locally thinning the membrane. Thus, although most of the literature focuses on the role of bacterial MSCs in osmotic adaptation and protection against hypoosmotic shock (36), MSCs have all the qualities to be nonspecific surface sensors, by homology to their role in contact sensing in fungi (103). MSCs are common in bacteria, with *E. coli* encoding several paralogs (MscK, M, L, S) (43, 191). The best-understood member of this family, MscL, is a homopentamer forming a large nonselective pore (3 nm) that gates in response to membrane tension as a result of mechanical force transmitted directly to the channel from the lipid bilayer (6, 213) (**Figure 3*d***). Membrane curvature traps MscL in its open conformation, while membrane thinning generates a hypersensitive MscL conformation (152).

3.3. Sensing of pH Changes Close to the Surface

Bacteria approaching a surface can experience significant pH changes (0.5–2 pH units; **Figure 3*c***) (75, 158). Using electrochemical sensors, researchers observed an acidification of the cell

surface interface over a period of 5 h postadhesion. In principle, bacteria could sense this acid pH shift at the cell surface interface by means of their Cpx two-component system (45, 135) or the PmrA/B system (150) and interpret this as a signal for surface adaptation. Surface near-acidification will also affect the proton motive force (75). Whether this affects surface sensing by the flagellar or pilus motors remains to be explored.

4. MECHANOTRANSDUCTION: SURFACE ADAPTATION PROGRAMS

In the previous sections, we focused on mechanisms and molecules that bacteria use to monitor specific mechanical cues during surface interaction. Processes of mechanotransduction include the conversion of mechanical stimuli into biochemical responses within cells. For gated MSCs, downstream signaling may simply involve a change in ion flux through the membrane. More complicated cellular responses require sophisticated mechanotransduction processes that eventually change the cell's physiological makeup or gene expression profile. In the following, we discuss known mechanisms of mechanotransduction considering the timescale of individual processes. We follow the surface adaptation process in chronological order, from rapid allosteric responses taking place within seconds and minutes after surface encounter to slower, generally transcriptional responses of surface adaptation in the timeframe of minutes to hours. As a general trend, bacteria (*a*) rapidly deploy adhesins to stabilize their attachment to surfaces, (*b*) stop flagellar rotation, (*c*) transcriptionally activate a dedicated surface program, and (*d*) transit into biofilm formation. Not unlike mammals, where second messengers play key roles in mechanotransduction involved in hearing, balance, or touch, bacteria make ample use of second messengers for mechanotransduction.

4.1. Role of c-di-GMP in Bacterial Surface Adaptation

The second messenger c-di-GMP is a master regulator of bacterial motile-to-sessile transitions (84, 204) and was shown to increase when bacteria are exposed to surfaces. In the following we summarize how c-di-GMP orchestrates these processes by regulating pili and flagella and by promoting adhesin and biofilm matrix production.

4.1.1. Rapid increase of c-di-GMP mediates adhesion.

Mechanical cues need to be converted into biochemical signals to elicit a cellular response. While this process was originally believed to be slow, recent studies indicated that surface

sensing by the flagellar motor triggers an increase of c-di-GMP within seconds (79, 104). The amount of c-di-GMP produced during initial surface encounter, although much lower than at later stages when cells develop mature biofilms, is sufficient to activate high-affinity effectors regulating the biogenesis of surface adhesins (79, 81, 104). In *P. aeruginosa*, this rapid surface response is sustained by a slower, T4P-dependent response, which contributes to further increasing the intracellular c-di-GMP concentration minutes to hours after surface contact (115, 169). Thus, prolonged exposure to a surface results in a gradual increase of c-di-GMP, a process that is likely sustained by the continuous surface-induced transcription of components of the c-di-GMP network (66) (**Figure 1**). In *C. crescentus*, surface sensing by the flagellar motor leads to the rapid production of c-di-GMP by the motor-associated diguanylate cyclase DgcB (79). Mechanosensing in this organism is reinforced by a T4P-dependent process, although in this case the link to c-di-GMP production is less clear (50).

4.1.2. c-di-GMP-mediated formation of pili and other surface adhesins.

Pilus assembly platforms are often present at the poles of rod-shaped bacteria but poorly active in planktonic cells (42). In *P. aeruginosa*, the percentage of piliated cells and the number of pili per cell strongly increase upon surface exposure (42, 104). Recent reports indicate that this process is mediated by c-di-GMP, which regulates pilus formation and activity at different levels and at different timescales, thereby stabilizing surface attachment (73) and inducing surface motility (23). In *P. aeruginosa*, T4P biogenesis is allosterically activated via different c-di-GMP receptors that appear to support different pilus functions. In its c-di-GMP-bound form, FimX directly interacts with the pilus-specific ATPase PilB to promote T4P elongation at the leading pole (82, 164). Unipolar localization of FimX regulates T4P dynamics to promote twitching motility (78, 90). Similarly, FimW promotes piliation of *P. aeruginosa* upon surface-mediated c-di-GMP upshift. However, in contrast to FimX, FimW was shown to promote surface adhesion rather than twitching motility. How FimW mediates pilus formation is unclear (104). Both FimW and FimX bind c-di-GMP with high affinity (50 and 90 nM, respectively), arguing that these proteins are activated immediately after surface contact when c-di-GMP levels are still low (81, 104). Assembly of T4Ps in *P. aeruginosa* allows the deployment of the surface adhesin PilY1 on the cell surface, which in turn activates the c-di-GMP-producing enzyme SadC, elevating the global intracellular concentration of c-di-GMP to sustain the surface program (115). A similar system was shown to control piliation in different *Xanthomonas* species. In this case c-di-GMP

binds to the FimX and PilZ proteins, which form a stable ternary complex with the PilB ATPase (67, 68). Although the precise role of c-di-GMP in controlling the FimX-PilZ-PilB complex is not clear, these protein-protein interactions likely modulate T4P biogenesis and twitching motility (49). c-di-GMP can also modulate pilus dynamics by directly interacting with motor ATPases. In *V. cholerae*, c-di-GMP interacts with the T4P motor protein MshE to promote MshA pilus biogenesis and near-surface motility (87, 170). Surface-tethered MshA pili drive a behavioral switch, during which *V. cholerae* starts orbiting close to the surface before irreversibly attaching cells and initiating microcolony formation (202). Similarly, c-di-GMP interaction with the pilus ATPase PilB2 regulates T4P assembly in the gram-positive pathogen *Clostridium perfringens* (71). *Vibrio* and *Clostridium* are evolutionarily distant organisms, arguing that c-di-GMP-mediated allosteric regulation of pilus biogenesis is a widely conserved mechanism. The link between c-di-GMP and pili is reinforced by the observation that c-di-GMP also regulates the expression of pilus genes. For example, in *Klebsiella pneumoniae*, transcription of type 3 fimbria genes is regulated by the c-di-GMP receptor MrkH (217). Similarly, in *Clostridium difficile* pilus genes are controlled by a c-di-GMP type II riboswitch (19) (**Figure 4*a***).

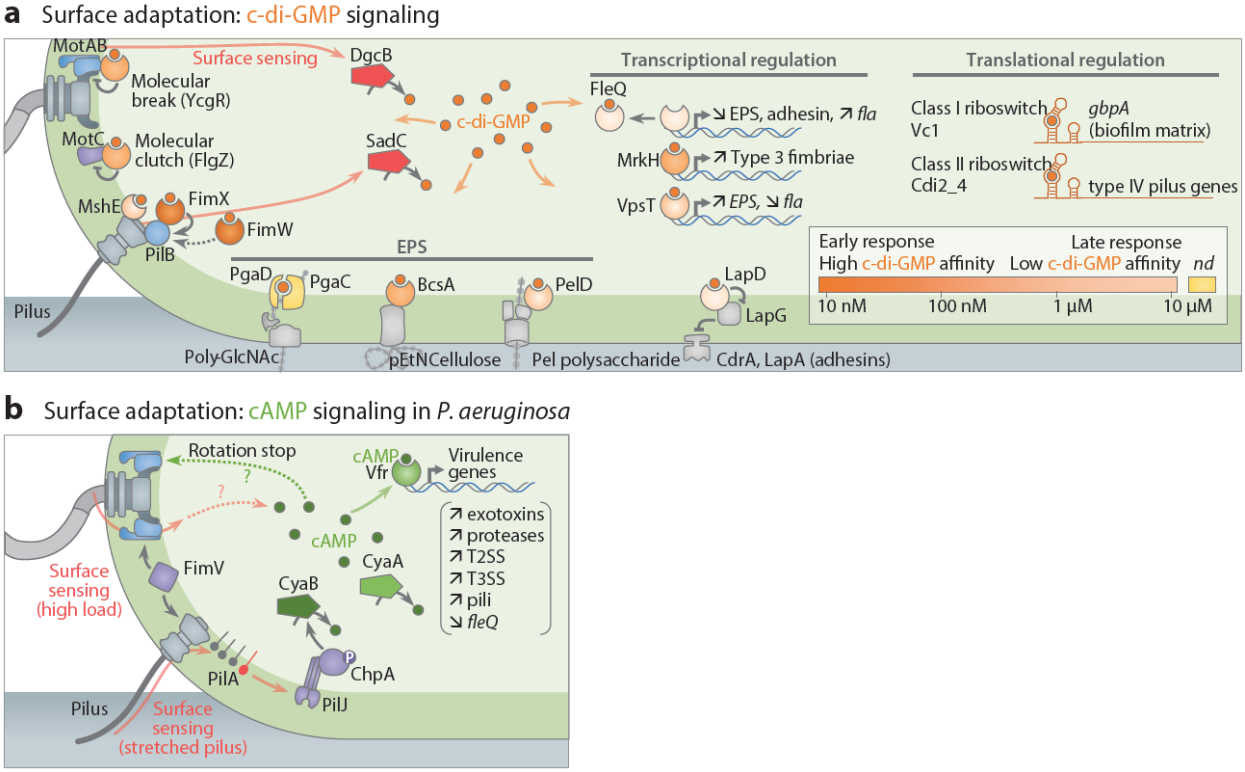

Figure 4 Overview of surface-sensing and adaptation mechanisms. (*a*) Mechanosensing by the flagellar and T4P

machineries lead to the production of c-di-GMP (e.g., by the enzymes DgcB in *Caulobacter crescentus* or SadC in *Pseudomonas aeruginosa*). At short timescales, c-di-GMP activates regulators of the T4P motor (FimX and FimW in *P. aeruginosa*) or directly activates pilus elongation ATPases (MshE in *Vibrio cholerae*). c-di-GMP tunes down flagellar rotation by means of molecular brakes (YcgR in *Escherichia coli*) or clutches (FlgZ in *Pseudomonas aeruginosa*). Biofilm formation is supported by activation of various EPS machineries (PgaCD and BcsA in *E. coli*, PelD in *P. aeruginosa*) and adhesins (CdrA stabilization by the c-di-GMP receptor LapD in *P. aeruginosa*). At longer timescales, c-di-GMP upregulates genes involved in adhesion and EPS synthesis by modulating transcription factors (inhibition of FleQ in *P. aeruginosa*, activation of MrkH in *Klebsiella pneumoniae*, and VpsT in *V. cholerae*). c-di-GMP-bound riboswitches regulate the translation of pili and biofilm matrix components (Vc1 in *V. cholerae*, Cdi2_4 in *Clostridium difficile*). (*b*) In *P. aeruginosa*, flagellum-based surface sensing mediates a stator-dependent cAMP increase. Additionally, stretched pilin subunits trigger the Pil/Chp pathway to activate the cAMP-producing enzyme CyaB. cAMP binding to the transcription factor Vfr drives *P. aeruginosa* surface-induced virulence. The hub protein FimV is required for both the flagellum- and the T4P-mediated surface response. Abbreviations: EPS, exopolysaccharide; GlcNAc, *N*-acetylglucosamine; pEtN, phosphoethanolamine; T2SS, type II secretion system; T3SS, type III secretion system; T4P, type IV pilus.

Apart from its central role in the assembly of pili and fimbriae, c-di-GMP also controls the formation of other surface adhesins. For example, *P. aeruginosa* biofilm formation and autoaggregation are promoted by the outer membrane surface adhesin CdrA (168). Expression of *cdrA* is mediated by the c-di-GMP receptor FleQ (20, 72). In addition, surface exposure of the CdrA adhesin is controlled at the posttranslational level via the c-di-GMP receptor LapD, which regulates CdrA degradation by the protease LapG (41).

4.1.3. Modulation of the flagellar motor by c-di-GMP.

Actively rotating flagella favor the detachment of surface-adherent bacteria (40). To stabilize their association with surfaces, bacteria need to pause flagellar rotation and to actively control their motility behavior. Many bacteria allosterically activate a flagellar brake or clutch to modulate motor behavior. This enables a reversible adaptation, in case conditions are unfavorable and the motor needs to be reactivated to leave the surface. Two different types of regulators were shown to tune the flagellar motor, flagellar breaks that jam the motor and flagellar clutches that disengage stators from the rotor (189). The flagellar brake mechanism was originally discovered in *E. coli* and relies on the c-di-GMP receptor YcgR (174). Upon binding of c-di-GMP, YcgR interacts with the motor protein MotA, resulting in decreased torque generation and reduced swimming speed (18, 77). YcgR was also shown to interact with the flagellar switch, resulting in a change of motor bias (51, 148). This observation is in line with other reports that link c-di-GMP directly to chemotaxis in different bacterial species (136, 145a, 172, 192, 219, 220). Interference with chemotaxis is thought to improve surface attachment by suppressing cell reorientations and thereby increasing surface residence times (190).

YcgR homologs are widespread in bacteria, and it is reasonable to assume that many bacteria use this protein to control flagellar motor behavior in response to changing c-di-GMP concentrations (34, 95). In *P. aeruginosa* the YcgR homolog FlgZ functions as a molecular clutch. For instance, *P. aeruginosa* harbors two stator sets, MotAB and MotCD, both of which are used for swimming motility in low-viscosity environments. Surface sensing is achieved specifically by MotAB (104, 179), while MotCD is required for swarming on moist surfaces or swimming under high-load conditions (101, 200). Upon binding c-di-GMP, FlgZ (a homolog of YcgR) specifically interacts with MotC and disengages MotC from the flagellar motor, thereby inhibiting high-torque flagellar motility on surfaces (5). Similarly, in *B. subtilis*, the YcgR homolog MotI acts as a molecular clutch, disengaging MotA from the rotor part of the flagellum (188).

Most bacterial species swim with bidirectional motors that respond to the external environment via the chemotaxis pathway. Binding of phosphorylated CheY (CheY~P) to the flagellar switch reverses motor rotation, enabling bacteria to accumulate in areas favorable for their growth and survival (160). In some species like *Rhodobacter sphaeroides*, which employs a single stop-start flagellar motor, CheY~P acts as a break to stop flagellar rotation (156). Interestingly, in *C. crescentus*, a close relative of *R. sphaeroides*, a subset of CheY-like (Cle) proteins interferes with the motor to enhance rapid surface attachment of motile cells (136). Unlike classical CheYs, Cle proteins are not activated by phosphorylation but instead bind c-di-GMP with high affinity and in response interact with the flagellar motor. Nesper et al. (136) proposed that these CheY-like proteins might be part of a regulatory loop controlling flagellar activity in response to a transient surface-mediated upshift of c-di-GMP. The observation that individual Cle components are required for the rapid surface response of *C. crescentus* suggested that they may form a positive-feedback loop by interacting with the mechanosensing device itself, thereby reinforcing the c-di-GMP upshift and surface adaptation (136).

4.1.4. Asymmetric division on surfaces balances attachment and spreading.

The initial increase of c-di-GMP following surface contact directs the assembly of surface adhesins in both *P. aeruginosa* and *C. crescentus* (79, 104). However, once attached to the surface, both species undergo asymmetric divisions generating offspring with distinct behavior. While one progeny remains surface attached, the other inherits an active flagellum from its mother and detaches from the surface after cell division is completed (40, 83) (**Figure 1**). In both

species, the differential behavior is determined by the asymmetric distribution of c-di-GMP during division. In *P. aeruginosa* the asymmetry is generated by the phosphodiesterase Pch, which is asymmetrically tethered to the flagellated pole (102, 104). During division, Pch segregates into the flagellated daughter, with its activity reducing the c-di-GMP concentration in this cell type, leading to pilus retraction and resumption of flagellar rotation (104). Similarly, in *C. crescentus*, c-di-GMP asymmetry is determined by the positioning of a diguanylate cyclase (PleD) and a phosphodiesterase (PdeA) to opposite poles of the dividing cell and their differential segregation during division (1, 2, 35). It was proposed that on the population level, this asymmetric behavior greatly enhances surface colonization by combining efficient surface attachment with effective dissemination and spreading on surfaces or host tissues (104). Asymmetric distribution of c-di-GMP upon cell division was also observed in *Salmonella enterica* serovar Typhimurium and in *K. pneumoniae* (35), arguing that a wide range of bacteria have evolved c-di-GMP-mediated asymmetric programs during division.

4.2. Role of cAMP in Bacterial Surface Adaptation

In bacteria the ubiquitous second messenger cAMP is best known for its role in growth control and catabolite repression (151, 221). But cAMP also plays an important role in virulence control in several bacterial pathogens (124). In *P. aeruginosa*, cAMP levels gradually increase with continued surface exposure and in response to mechanical cues, resulting in the upregulation of a range of virulence factors (107, 154). While such a strategy makes sense for an opportunistic pathogen that assails by colonizing and damaging tissue surfaces and epithelial linings, it remains unclear how common this mechanism is in other bacterial pathogens.

4.2.1. Surface-induced cAMP controls virulence factor expression in *P. aeruginosa*.

In this organism, a surface-specific increase of cAMP is mediated by the T4P machinery. Retraction of tethered pili is sensed through the Chp chemosensory system (154), which in turn upregulates intracellular cAMP levels (60). Persat and collaborators (154) proposed that stretched PilA subunits specifically interact with the chemoreceptor-like protein PilJ, transducing mechanical signals to the cytoplasm by controlling the activity of the receptor-coupled kinase ChpA (59). ChpA not only increases surfaces sensitivity by stimulating T4P activity (154) but also activates the adenylate cyclase CyaB (14, 60). The resulting cAMP upshift induces the expression of virulence genes (exotoxins, proteases, type II and III secretion systems, pilus

genes, etc.) through the cAMP-dependent transcription factor Vfr (38, 58) (**Figure 4*b***). In parallel, cAMP contributes to *P. aeruginosa* surface adaptation by downregulating FleQ (a transcription factor stimulating flagellar genes and inhibiting exopolysaccharide genes) and upregulating adhesion factors (*pilY1* and pilus genes). More recently, cAMP levels were shown to increase in response to increased flagellar load, leading to a complete flagellar arrest within 5–15 min after surface tethering (179). Interestingly, the cAMP response appears to be confined to a specific time window postadhesion. An increase of cAMP is detectable (using transcriptional reporters) 30 min after surface contact, peaks 2–3 h after landing, and drops to basal planktonic levels at 4 h postadhesion (115, 154). After this window of acute virulence, *P. aeruginosa* switches to a chronic infection program that is driven by increasing levels of c-di-GMP (see below). It is tempting to speculate that the window of cAMP-mediated virulence closes simultaneously with the initial response of the innate immune system, a process that *P. aeruginosa* counters by transiting toward a protective biofilm lifestyle.

4.3. Long-Term Surface Adaptation

Prolonged exposure of bacteria to surfaces prompts major physiological changes that eventually lead to a sessile lifestyle and the initiation of biofilm formation (54). This behavioral transition was studied primarily on the transcriptional level (4). Changes at shorter timescales and in spatially defined subcompartments were also observed but are experimentally more challenging (15, 94, 133, 182).

4.3.1. Surface-induced c-di-GMP regulates exopolysaccharide synthesis.

The long-term adaptation to surfaces includes the synthesis of exopolysaccharides (EPSs) to encapsulate expanding bacterial communities in a protective matrix (203). As outlined above, surface exposure triggers a gradual increase of the intracellular c-di-GMP concentration, which in turn regulates the production of EPSs and other matrix components at the transcriptional, translational, and posttranslational level (84) (**Figure 1, 4*a***). For example, c-di-GMP allosterically activates two different EPS machineries in *E. coli*. By mediating the interaction and activity of two inner membrane proteins, PgaC and PgaD, involved in the polymerization of *N*-acetylglucosamine moieties, c-di-GMP stimulates the synthesis of poly-*N*-acetylglucosamine (K_{cat} = 62 nM) (186). Similarly, c-di-GMP binding releases the autoinhibited state of the BcsA polymerase (K_d = 0.4 μM), thereby enhancing its activity in synthesizing phosphoethanolamine

cellulose by more than 20-fold (130, 196). In *P. aeruginosa*, binding of c-di-GMP to PelD activates the Pel EPS synthesis machinery (108) ($K_d \approx 1$ μM). At increasing levels of c-di-GMP, the genes encoding components of the Pel and Psl EPS machineries are derepressed through the inhibition of the c-di-GMP-dependent transcriptional repressor FleQ ($K_d > 4$ μM) (72, 121). In *V. cholerae*, c-di-GMP activates the transcription factor VpsT ($K_d = 3.2$ μM) to induce EPS production and represses flagellar genes (16, 99). In *Vibrio vulnificus*, c-di-GMP promotes the transcription of *cabA*, coding for a structural protein of the extracellular matrix required for biofilm maturation (147). On the translational level, the class I c-di-GMP riboswitch Vc1 is located upstream of the adhesion factor GbpA in *V. cholerae* and positively regulates its translation in response to c-di-GMP (88).

4.3.2. Exopolysaccharide inhibits flagellar rotation.

Apart from its adhesive and protective function in surface-grown biofilms, matrix EPS can also directly impact flagellar function. In *S. enterica*, cellulose produced in high-c-di-GMP conditions inhibits flagellar rotation (222). Based on observations that the expression of flagellar genes and flagellar assembly are not affected, cellulose was proposed to function as a wheel lock to sterically block flagellar rotation from the outside of cells (214). A similar phenomenon was observed in *B. subtilis*, where EpsE, a glycosyl transferase involved in EPS biosynthesis, acts inside bacterial cells as a molecular clutch to stop flagellar rotation (65).

5. CLOSING REMARKS

Bacteria are well equipped to thrive on all kinds of surfaces and exploit such environments either for nutritional reasons or as a means of protection. Our review highlights several examples of surface-mediated behavior and the responsible sensory mechanisms. Despite the obvious relevance of this process for bacterial growth and survival, only a few examples describing mechanisms of mechanosensation are documented. The Berg lab has pioneered studies on flagellar motor rearrangements at high-load conditions (109). Recent work on pilus-based mechanosensation, although much less understood, has significantly advanced mechanistic insight into these complex processes. It will be exciting to witness future discoveries on flagellum-based sensing, on pilus motor rearrangements, and on the sensing of pilin deformation by tensile force. The experimental approaches necessary for such studies are often technically

challenging. Recent advances in imaging technologies [cryo–electron microscopy (210), interferometry (193), microfluidics (144), optical and magnetic tweezers (206), and force measurements (30)] combined with genetic tools and careful examinations at short timescales will hopefully unlock some of the secrets of bacterial mechanosensation. On a molecular level we will need to address how force modulates the function of specific proteins or protein complexes and what the consequences are in terms of signaling. Detailed structural information of relaxed and mechanically induced conformations of the eukaryotic piezo mechanosensors (113, 211), the MSC McsL (7), or the catch-bond behavior of FimH (106) could serve as blueprints.

In the second part of this review we provide an overview of the mechanisms involved in mechanotransduction, explaining how bacteria adapt to their new surface environment. The second messengers c-di-GMP and cAMP have emerged as regulatory masterminds orchestrating attachment of bacteria to and growth and behavior on surfaces. In the future, it will be important to understand whether and how the activity of different surface sensors like flagella and pili is coordinated to integrate mechanical signals and how their downstream signaling components interact with each other. For example, in *P. aeruginosa*, cAMP- and c-di-GMP-dependent systems are induced simultaneously in response to surfaces and include mechanosensory feedback on motility and adhesion. But we do not understand how they cross talk with each other to optimally coordinate the cellular responses on different timescales. Finally, it will be important to gauge the relevance of some of these processes for the fitness and behavior of bacteria in the environment or in human and other animal hosts. For example, how important is the ability to respond to surfaces for pathogens to induce virulence and successfully infect different host tissues? We are only beginning to understand the mechanisms driving the interactions of bacteria with different surfaces. It will require experimental studies at the interface between biology and physics to shed light on the ubiquitous properties that allow bacteria to colonize surfaces so effectively.

DISCLOSURE STATEMENT

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