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REVIEW

SUBJECT COLLECTION: MECHANOTRANSDUCTION

Bacterial mechanosensing: the force will be with you, always

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ABSTRACT

Whether bacteria are in the planktonic state, free-swimming or free-floating in liquid, or in the biofilm state, sessile on surfaces, they are always subject to mechanical forces. The long, successful evolutionary history of bacteria implies that they are capable of adapting to varied mechanical forces, and probably even actively respond to mechanical cues in their changing environments. However, the sensing of mechanical cues by bacteria, or bacterial mechanosensing, has been under-investigated. This leaves the mechanisms underlying how bacteria perceive and respond to mechanical cues largely unknown. In this Review, we first examine the surface-associated behavior of bacteria, outline the clear evidence for bacterial mechanosensing and summarize the role of flagella, type-IV pili, and envelope proteins as potential mechanosensors, before presenting indirect evidence for mechanosensing in bacteria. The general themes underlying bacterial mechanosensing that we highlight here may provide a framework for future research.

KEY WORDS: Bacterial mechanosensing, Rotating flagella, Retraction of type-IV pili, Envelope proteins

Introduction

To survive and develop, eukaryotic cells have to adapt to a host of mechanical cues from the environment, such as matrix rigidity, substrate topography and fluid flow. Numerous studies have been conducted to understand how eukaryotes sense and respond to mechanical cues, thus using both physical and biological perspectives to establish the field of cellular mechanosensing (Cheng et al., 2017; Luo et al., 2013; Wang and Thampatty, 2006). Mechanosensing is identifiable when there is a biological response – e.g. signaling, change in gene expression, adaptation of protein function – in response to a mechanical cue, and varying the mechanical cue produces a change in the biological response.

Bacteria are well-known to respond to some types of mechanical cues; internal and external mechanical cues help to set bacterial size and shape (Si et al., 2015; Tuson et al., 2012) and changes in membrane tension can be responded to by mechanosensitive channels in the bacterial membrane (Booth, 2014; Haswell et al., 2011). Nevertheless, far more is known about eukaryotic mechanosensing than is known about whether, and how, bacteria respond to external mechanical cues by actively regulating biological processes. In part, this may be because bacterial mechanosensing has been under-investigated. However, bacteria have to negotiate and adapt to a wide variety of mechanical environments, in which mechanical characteristics are dynamic, not static (Persat et al., 2015b). Questions of mechanosensing are especially, although not exclusively, interesting for bacteria that are attached to surfaces. In nature, most bacteria live on surfaces and experience more mechanical stresses, and over a greater range of values, than do bacteria in fluid suspension. Furthermore, many surface-attached bacteria develop into biofilms, interacting communities of microbes bound together in a matrix made up of polymers and proteins. Biofilm development is a regulated, sequential process; here, a system consisting of many bacteria encounters mechanics that are different from those encountered by non-biofilm bacteria (Trejo et al., 2013). It is plausible, although it has not yet been demonstrated, that changes in the mechanical environment experienced by bacteria in biofilms could serve as regulatory cues or checkpoints.

Recent work has started to reveal cases of bacterial mechanosensing. For two specific types of bacteria (*Escherichia coli* and *Pseudomonas aeruginosa*) there is direct evidence for mechanosensing in the form of a biological activity that has been measured to vary specifically with varying mechanical input (Alsharif et al., 2015; Chawla et al., 2017; Lele et al., 2013; Rodesney et al., 2017). For *E. coli*, *P. aeruginosa* and many other species, there is also a great deal of indirect evidence for mechanosensing in that a biological response is triggered when bacteria encounter a mechanical cue. Such indirect evidence largely takes the form of surface-sensing or attachment-dependent behaviors (O'Toole and Wong, 2016; Tuson and Weibel, 2013). Given the widespread importance of mechanosensing in eukaryotic cells and the very large body of indirect evidence for bacterial mechanosensing, it is very likely that mechanosensing is widespread among many species of bacteria.

In this Review, we give a brief overview of what is already known about surface-sensing and attachment-dependent behavior of bacteria. Then, we briefly summarize the best-studied bacterial mechanosensory elements and the few cases, for which mechanosensing has been clearly shown to occur for bacteria, wherein a mechanical cue leads directly to a biological response. Notably, most of these cases involve a bacterial motility appendage driven by a motor, suggesting that motor response may be a widespread theme in bacterial mechanosensing. Blocking the active motion of bacterial appendages results in the increase of mechanical load on the associated motors, and subsequently, the mechanical signal is presumably transduced into some type of electrical or chemical signals in the cells. Therefore, we next discuss indirect evidence for mechanosensing that has been provided by studies of motility appendages and their motors. Finally, we point out other findings that have not, to our knowledge, been explicitly linked to mechanosensing, but we believe these findings provide additional indirect evidence for bacterial mechanosensing.

Setting the stage

Size and force regime

Bacterial cell bodies are typically on the order of 1 µm in extent in any direction, and therefore any mechanosensing that requires the entire cell surface, or a significant fraction thereof, ought to be sensitive to mechanical cues or variation on the scale of ∼1 µm.

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However, bacterial appendages (such as the flagellum and pili shown in Fig. 1i), which have been implicated in mechanosensing and can attach to materials with their tips, are ∼5–8 nm in diameter in the case of pili (Craig et al., 2004) and ∼20 nm in diameter in the case of flagella (Macnab, 2003); this suggests that mechanosensing that uses flagella or pili might be sensitive to mechanical cues or variation on length scales much less than a micron. However, while they are thin, both flagella and pili can have lengths equal to or greater than that of the bacteria themselves; this suggests that these appendages might be capable probes of mechanical cues or variations occurring over length scales greater than a micron. Membrane proteins (see inset to Fig. 1i), which have also been implicated in mechanosensing, are also characteristically a few nanometers in size. This may suggest that membrane proteins would be sensitive to mechanical cues that occur on the length scale of nanometers, if they have a portion that extends past the cell envelope to act as a localized mechanosensor. However, this would not be the case if, as seems likely to us, membrane proteins require deformation of the cell envelope to activate mechanosensing. Not enough is known about bacterial envelope proteins to make declarative statements about this.

Swimming bacteria, driven by rotating flagella, typically live in conditions with a low Reynolds number, i.e. viscous forces dominate over inertial forces (Purcell, 1977). In contrast, twitching bacteria, which move along a surface powered by extension and retraction of pili, can have ballistic characteristics on short timescales, indicating that on some timescales they live in an inertia-dominated scenario with a high Reynolds number (Bisht et al., 2017). A single pilus motor can generate a force greater than 100 pN (Maier et al., 2002), and a single flagellum motor can generate a torque of a few thousand pN nm (Berg, 2003; Lowe et al., 1987).

Surface sensing

Bacteria are active swimmers rather than passive colloid particles. When they contact surfaces, adhesins allow them to attach (Jarrell et al., 2011). Many different types of bacteria have adhesins, and many bacteria express more than one type of adhesin (Cooley et al., 2013; Jarrell et al., 2011). Adhesin types include bacterial appendages (pili, flagella and curli), proteins and extracellular polymers (Fig. 1). The diversity of these attaching mechanisms, and the redundancy provided by having multiple adhesin types for a single organism, indicate that the ability to attach to surfaces is critical for bacteria. A stable attachment onto surfaces is also important for most of the cases of bacterial mechanosensing that are demonstrated by the direct evidence discussed below (Alsharif et al., 2015; Rodesney et al., 2017), and is likely to be important for many more.

Many species of bacteria initiate biological responses when they have attached to a surface (Belas and Suvanasuthi, 2005; Gode-Potratz et al., 2011; Li et al., 2012). One of the major changes in a bacterium's environment when it transitions from being suspended in a fluid to being attached to a substrate is a change in the mechanical characteristics of the environment. Thus, the widespread observation that bacteria initiate signaling and undergo phenotypic changes – i.e. the bacteria show a biological response – upon coming out of fluid suspension to attach to a solid surface, is itself suggestive of mechanosensing.

Attachment-dependent behavior

Perhaps the most striking attachment-dependent behavior of bacteria is biofilm development. The sessile bacteria embedded in biofilms have different patterns of gene expression, metabolism and many other properties, than their planktonic (i.e. free-swimming or

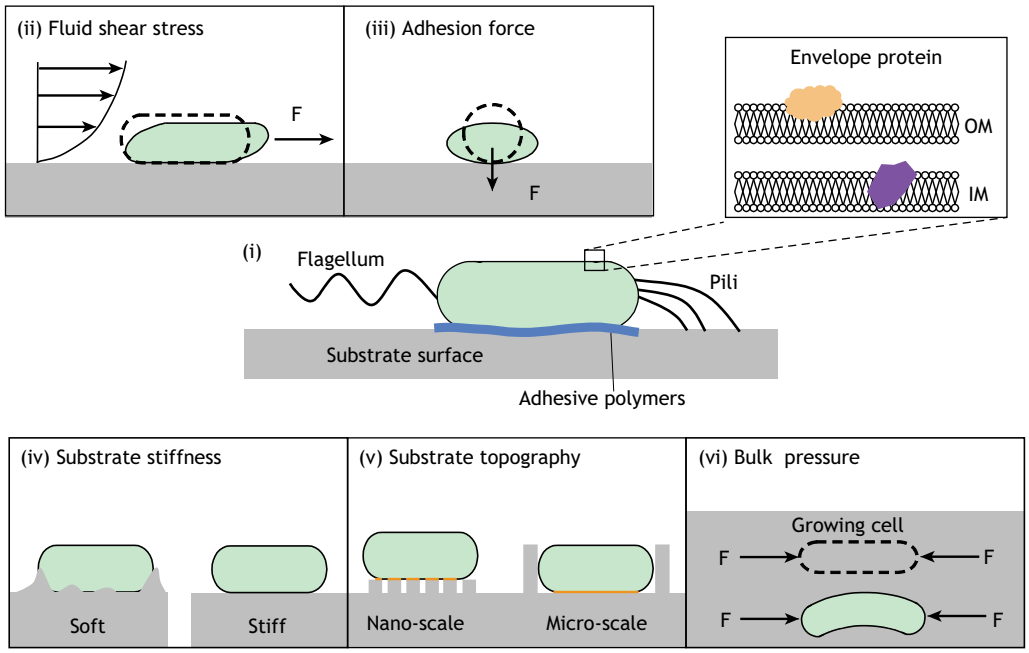

Fig. 1. The mechanics bacteria could experience upon attaching to a surface. (i) Adhesins, including pili, flagella, envelope proteins (see inset; OM, outer membrane; IM, inner membrane) and extracellular polymers allow bacteria to attach on surfaces. (ii) The flow of surrounding fluid generates shear force on surface-attached bacteria. The pili and adhesive polymers help to prevent bacterial detachment from the surface; the cell envelope may also deform due to shear (exaggerated in this schematic for visibility). (iii) The bacterial cell is subjected to adhesion forces (e.g. polymer-mediated adhesion, electrostatic or van der Waals forces) from the surface, leading to a deformation of the cell envelope (exaggerated in this schematic for visibility). (iv) Bacteria encounter substrates of different stiffnesses that deform differently in response to adhesion forces; this can impact the surface area of the bacterial cell envelope that is in contact with the substrate. (v) Surface topographies may affect bacterial adhesion. (vi) When bacteria are embedded in a bulk material, growing cells experience pressure that depends on the modulus of the surrounding material.

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free-floating) counterparts (Flemming et al., 2016; Wang et al., 2015b).

Although our recent work has shown that biofilms need not necessarily develop only from single cells (Kragh et al., 2016), the vast majority of studies have focused on the standard model of biofilm development, which begins with planktonic bacteria approaching and adhering to surfaces (Joo and Otto, 2012; O'Toole, 2003). This is the initial step for bacteria to switch from a motile to a sessile life.

Biofilm formation is regulated by a variety of signals that control modulations of microbial biology (O'Toole et al., 2000). Besides biological and chemical cues, there are also potential mechanical cues present during biofilm formation (Fig. 1). During biofilm initiation, swimming bacteria may detect mechanical stimuli through initial surface contact and, after attaching to surfaces, bacteria experience an adhesion force from the surface, substrate stiffness and topography, as well as shear that can vary with surface motility and with changing fluid flow. Macroscopic biofilms exhibit viscoelastic behavior, and external force – e.g. from shear flow – can cause structural deformation and changes in the mechanics of biofilms over time (Guélon et al., 2011; Karimi et al., 2015).

A second type of attachment-dependent behavior is surface motility. Bacteria may move laterally on the surfaces to which they are attached using several modes of motility: swarming, twitching, gliding and sliding (Harshey, 2003) (Fig. 2). Swarming is the collective movement of cell clusters, powered by rotating flagella, and usually takes place on soft agar plates in the laboratory (Kearns, 2010). Twitching is a flagella-independent process, in which single cells or colonies move by pulling themselves along via the extension and retraction of type-IV pili (Merz et al., 2000). Gliding is an active surface-motility mode found in some bacterial species that does not involve pili (Gibiansky et al., 2013). Finally, sliding or spreading is surface translocation powered by the expansive forces that result from cell growth, which does not require an active motor (Harshey, 2003). Of the surface motility modes identified, the passive modes of sliding and spreading appear the least likely to be linked to bacterial mechanosensing, which generally involves functions of active motors and envelope proteins, as discussed below. Sliding or spreading does not use active motors and there is no good reason, to our knowledge, to believe that forces associated with these very slow motility modes will have any appreciable effect on envelope proteins beyond that of surface adhesion itself.

Cast of characters

Envelope proteins and motility appendages driven by motors

In the little that is known about bacterial mechanosensing, a widespread theme is the importance of motility appendages and their associated motors.

Bacterial flagella are rotating, rigid helices that propel individual bacteria to swim when surrounded by liquid, or cell clusters to swarm on surfaces (Harshey, 2003; Kearns, 2010). The flagellar motor is composed of membrane-embedded stators and a transmembrane rotor (Berg, 2003) (Fig. 3A). Flagellar stators employ an ion-motive force, typically a H^+- or Na^+-gradient-generated motive force (proton motive force, PMF or sodium motive force, SMF) to drive the rotor (Chevance and Hughes, 2008). Some bacteria contain more than one set of stator proteins. For instance, *P. aeruginosa* uses two H^+-powered stator sets – MotAB (made of MotA and MotB proteins) and MotCD (made of MotC and MotD proteins) (Kuchma et al., 2015), and *Shewanella oneidensis* has both a H^+-powered stator (MotAB) and a Na^+-powered stator (PomAB, made of PomA and PomB proteins) (Paulick et al., 2015).

Type-IV pili are retractable helical filaments. They elongate by polymerization, adhere to a substrate, and retract by depolymerization of the major pilin subunit (PilA). Here, elongation and retraction are powered by the two types of cytoplasmic ATPases, PilF/B (PilF and PilB are orthologs) and PilT, respectively (Fig. 3B). The successive extension and retraction of type-IV pili drive bacterial twitching motility, independent of flagella (Merz et al., 2000).

In addition to the importance of motor proteins, envelope proteins have also been identified as important for bacterial mechanosensing. The bacterial cell envelope is composed of the inner cell membrane and the cell wall, as well as, in the case of Gram-negative bacteria, the outer membrane. Envelope proteins are embedded directly in the bacterial cell envelope and not incorporated in motility appendages or motors. If the cell envelope is distorted, either as a result of adhesion to the surface (Fig. 1ii) or by some other force, such distortion could be transmitted to a mechanosensitive envelope protein, thereby providing a mechanical signal that is transduced by the protein into a biological signal. In this context, it is noteworthy that the mechanical stiffness of bacterial cells has been found to be tightly regulated (Box 1).

In *E. coli*, the CpxA–CpxR two-component system is thought to sense the deformation of the cell membrane (Fig. 4C). When membrane stress increases, the outer membrane protein NlpE

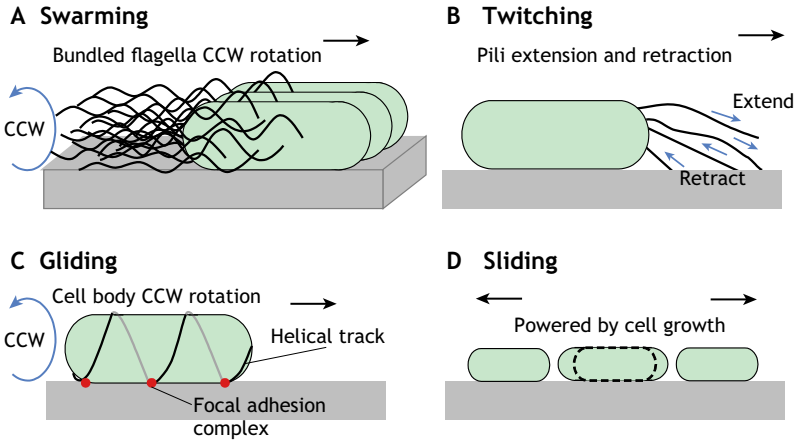

Fig. 2. Modes of bacterial motility on a surface. (A) Swarming motility is a multicellular movement powered by flagella that are rotating counterclockwise (CCW). (B) Twitching motility is a surface movement exerted either by a single bacterium or groups of bacteria. Twitching is powered by extension and retraction of type-IV pili. (C) Gliding motility is driven by motor proteins, which move within the cell along a helical track. The motors remain in fixed positions with respect to the substrate, thereby forming focal adhesion complexes, which help to propel the cell body forward using a CCW rotation. (D) Sliding motility is a passive surface movement that is driven by cell growth.

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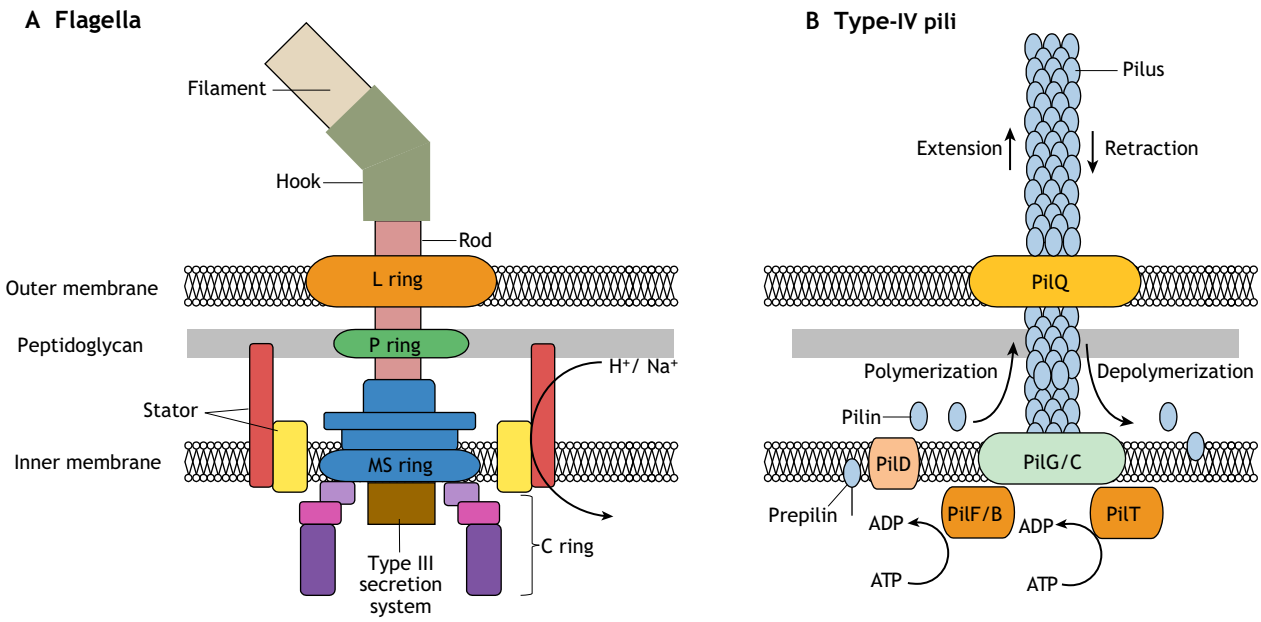

Fig. 3. Schematic illustration of bacterial motility appendages that have been implicated in mechanosensing. (A) The flagellum consists of a basal body, hook and filament. The basal body is embedded in the inner and outer membranes and is composed of L rings, P rings, MS rings and C rings. The hook connects the helical filament to the basal body. The flagellar rotation is powered by a H^+- or Na^+-driven motor, which consists of a rotor (C ring) and stators. The scheme has been adapted from Pallen and Matzke (2006) with permission from Elsevier. (B) The type-IV pilus filament is mainly composed of pilin subunits called major pilins (PilA in *P. aeruginosa*). Prepilin is cleaved by PilD, and the processed pilin subunits are assembled by PilG/C into the pilus filament, which emerges from the bacterium body through PilQ. The ATPase proteins PilF/B and PilT mediate pilus polymerization (extension) and depolymerization (retraction), respectively. The scheme has been adapted from Maier and Wong (2015) with permission from Springer Nature.

activates the first component CpxA, an inner membrane protein. Next, CpxA undergoes autophosphorylation and transfers its phosphate groups to the cytoplasmic response regulator protein CpxR, the second component in the pathway. Then, phosphorylated CpxR activates transcription of target genes (Otto and Silhavy, 2002).

Furthermore, recent work indicates that *P. aeruginosa* may use the envelope protein PilY1 as a mechanosensor for surface sensing (Luo et al., 2015; Siryaporn et al., 2014). PilY1 has some structural resemblance to the mechanically sensitive von Willebrand factor A (VWFa) domain (Kuchma et al., 2010). The VWFa domain is widely found in higher eukaryotes and VWF proteins can be deformed by shear forces (Colombatti and Bonaldo, 1991; Springer, 2014). The VWFa domain of PilY1 in *P. aeruginosa* has also been found to play an important role in surface-associated behaviors, e.g. swarming motility (Kuchma et al., 2010).

The above envelope proteins in *E. coli* and *P. aeruginosa* are often found to be linked to bacterial virulence against host cells (Shimizu et al., 2016; Siryaporn et al., 2014), which will be discussed later.

Box 1. Regulation of the mechanics of bacteria

The stiffness of the bacterial cell envelope will determine how much it is deformed by adhesion and/or contact forces, and, therefore, impacts the conformational change(s) in any mechanosensory envelope proteins. Recent work has shown that bacterial cell stiffness is tightly regulated by many interacting genes (Trivedi et al., 2018). This is consistent with the idea that appropriate deformation of the cell envelope and embedded envelope proteins, leading to appropriate mechanosensing and response, could provide a selective advantage for bacteria under evolutionary pressure – e.g. by promoting the development of biofilms that could help protect bacteria from predators (Joubert et al., 2006; Matz et al., 2008).

Chemical products

To date, many of the identified biological responses of bacteria to mechanical inputs have been in the form of either increased intracellular cyclic diguanylate monophosphate (c-di-GMP) or increased production of virulence factors (Alsharif et al., 2015; Rodesney et al., 2017; Siryaporn et al., 2014). C-di-GMP is a second messenger that is widespread among many types of bacteria. It controls, among other things, surface-associated behaviors such as biofilm formation (Jenal et al., 2017). Generally, bacteria in biofilms have a higher c-di-GMP concentration than planktonic counterparts, as bacteria use an elevated c-di-GMP level to stimulate the production of adhesins and exopolysaccharide matrix in biofilms (Hengge, 2009). In surface-associated *P. aeruginosa*, when the c-di-GMP level is high, the MotAB stator (which cannot support swarming motility) can displace the MotCD stator (which promotes swarming motility) from the motor, thereby repressing bacterial swarming and promoting biofilm formation on surfaces (Baker et al., 2016; Kuchma et al., 2015).

Virulence factors are molecules produced by microbes that help them to invade and injure host cells; examples are pyocyanin and hydrogen cyanide generated by *P. aeruginosa* (Siryaporn et al., 2014). Both pyocyanin and hydrogen cyanide can easily penetrate biological membranes. Pyocyanin has been shown to inactivate catalase and disrupt calcium homeostasis in human epithelial cells, and cyanide can inhibit cellular aerobic respiration (Lau et al., 2004; Lenney and Gilchrist, 2011). Notably, both c-di-GMP and another widespread, multifunctional second messenger with many roles, cyclic adenosine monophosphate (cAMP), are involved in virulence regulation (McDonough and Rodriguez, 2012).

Action: clear-cut cases of bacterial mechanosensing

Below, we will discuss the direct evidence for bacterial mechanosening; we consider direct evidence to be cases in which

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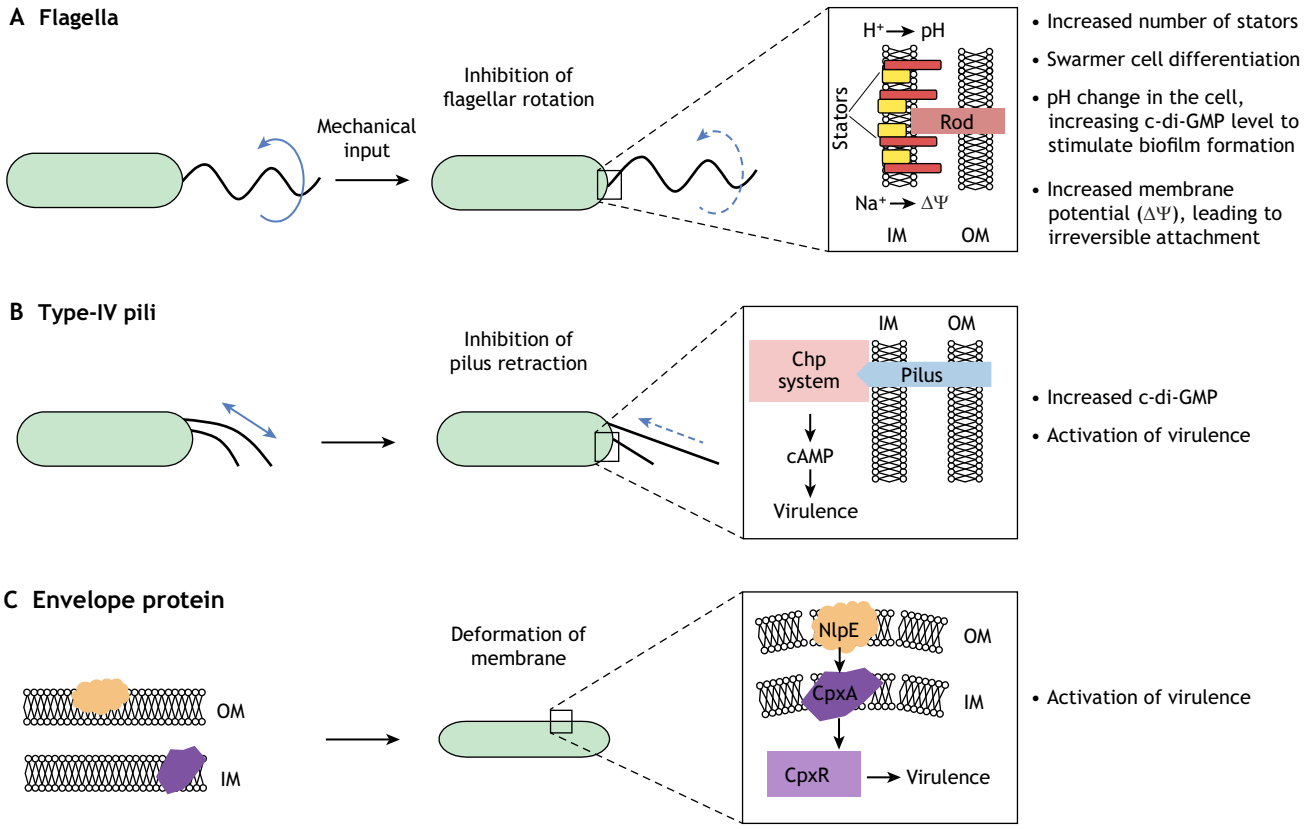

Fig. 4. Bacterial mechanosensing elements and potential pathways and responses to mechanical inputs. (A) Flagella. When bacteria are swimming, increases in the viscosity of the liquid environment or contact with substrate surfaces inhibit flagellar rotation. This may increase the amount of stators in the flagellar motor to trigger swarmer cell differentiation and interrupt the H^+ or Na^+ ion flux through the stators to stimulate or initiate biofilm formation. (B) Type-IV pili. Upon surface contact, the inhibition of pilus retraction generates tension in the pili, which is sensed by the Chp system, leading to cAMP-dependent upregulation of virulence. Shear stresses applied on the surface-attached bacteria also generate tension in the pili during pilus retraction, which elevates the level of c-di-GMP. (C) Envelope protein. The adhesion force exerted by the surface and the shear stress yield a deformation of the bacterial cell membrane, which is sensed by the outer membrane protein NlpE, triggering the signal transduction through the CpxA–CpxR two-component system. CpxR is then involved in virulence activation. OM, outer membrane; IM, inner membrane.

changes of biological responses in bacteria have been observed upon varying mechanical input. When planktonic bacteria are swimming in a fluid, changing the viscosity of the liquid environment will vary the mechanical load on rotary flagellar motors (Fig. 4A). *E. coli* can adapt to changes in the torque required to swim at a given speed by adding new force-generating units (i.e. stators) onto the motor (Chawla et al., 2017; Lele et al., 2013; Tipping et al., 2013). Another group found that when the viscosity of the fluid environment is increased, more *Proteus mirabilis* cells elongate to lengths greater than 35 µm – this cell length is used to define 'swarmer' cells in this study (Belas and Suvanasuthi, 2005). In addition, inhibition of the rotation driven by flagellar motors, as by increasing the mechanical load imposed by the viscosity of the fluid environment, triggers differentiation into swarmer cells (Belas and Suvanasuthi, 2005). These examples show that some bacteria can sense changes in the viscosity of the liquid environment by sensing changes in the external mechanical load that acts to inhibit the rotation of their flagella.

For surface-associated *P. aeruginosa*, mechanical shear, arising from the bacterial twitching motility combined with friction-like adhesion with the surface, was found to act as a mechanical cue to increase production of the intracellular signal c-di-GMP (Rodesney et al., 2017), which can initiate biofilm formation (Jenal et al., 2017). Increasing shear (from 0 to 0.01 Pa) by varying the speed of fluid flow over surface-attached *P. aeruginosa* also correlates with increasing levels of intracellular c-di-GMP (Rodesney et al., 2017). For this mechanosensing of mechanical shear, both type-IV pili with a functional retraction motor and the envelope protein PilY1 are required (Rodesney et al., 2017), which suggests that the pilus motor and the envelope protein PilY1 may be mechanosensory elements involved in the sensing of shear by surface-associated *P. aeruginosa* (Fig. 4B). The mechanical shear yields a tension in the type-IV pili or a deformation of the bacterial cell envelope, which might be then transduced into an elevated level of c-di-GMP.

Bacterial mechanosensing of the adhesion force exerted by the surface has been found in enterohemorrhagic *E. coli* (EHEC) (Alsharif et al., 2015). EHEC causes diseases through the expression of virulence factors, and some of these factors are controlled by a genomic pathogenicity island called the locus of enterocyte effacement (LEE). EHEC attached to a host cell or on glass coated to be more adhesive to EHEC has an increased adhesive interaction with these surfaces compared to bare glass. Alsharif et al. (2015) showed that such an increased adhesive interaction between EHEC and surfaces leads to an increase in LEE activation, i.e. an increased virulence, and they also found that enhanced shear (0.01–1 Pa) applied by varying the speed of fluid flow over the surface-associated EHEC could further elevate LEE activation. A subsequent study found that bacterial attachment to surfaces allows NlpE in EHEC cells to upregulate the type III secretion system that is encoded by LEE genes, and that CpxR binds to the *lrhA* promoter region, which encodes the transcriptional regulator LrhA to regulate the expression

of LEE genes (Shimizu et al., 2016). We therefore speculate that the envelope protein system NlpE–Cpx may be a mechanosensor that allows EHEC to sense adhesion force and shear, leading to the subsequent transduction of these mechanical signals to the biological response of increased virulence.

Foreshadowing: indirect evidence for bacterial mechanosensing

Responses to initial contact with surfaces

Flagellar motors

It seems very likely that flagellar rotary motors may have a mechanosensory role, in addition to the cases of *E. coli* and *P. mirabilis* swimming in variable-viscosity fluids we discussed above (Chawla et al., 2017; Lele et al., 2013; Tipping et al., 2013). Similar to increasing fluid viscosity, contact with surfaces will obstruct or inhibit flagellar rotation – this presents a possible mechanism for surface sensing and the initiation of surface-associated behavior (Fig. 4A). Indeed, there is good experimental evidence that this may be the case for *Vibrio parahaemolyticus* (Gode-Potratz et al., 2011; McCarter et al., 1988), *Bacillus subtilis* (Cairns et al., 2013), *Caulobacter crescentus* (Hug et al., 2017) and *P. mirabilis* (Belas and Suvanasuthi, 2005).

What mechanism(s) underlie this type of putative mechanosensing process is unclear. One possibility of sensing the external mechanical load acting on rotating flagella is by surface contact; this might increase the number of stators in the flagellar motor, as discussed above (Chawla et al., 2017; Lele et al., 2013; Tipping et al., 2013). A higher number of stators then may allow bacteria to sense surfaces and to initiate swarming across soft solid surfaces (Tipping et al., 2013).

The disruption of PMF and/or SMF owing to the obstruction of flagellar rotation seems another likely mechanism. This hypothesis can be supported by the following findings. It was reported that the surface contact of *C. crescentus* causes a change in the H^+ flux through the flagellar stators; therefore, the transient pH change inside the bacteria cell might be subsequently sensed by diguanylate cyclase DgcB, which can stimulate the production of c-di-GMP to promote biofilm formation (Hug et al., 2017). Similarly, it was also found that after *Vibrio cholera* cells attach to surfaces, the inhibited flagellar rotation interrupts the decreased Na^+ flow through the flagellar motor, leading to an increase in membrane potential ($\Delta\Psi$), which might initiate the transition of surface-associated bacteria from reversible to irreversible attachment (Van Dellen et al., 2008).

Pilus motors

Type-IV pili, and specifically their retraction motors, seem likely to be involved in bacterial mechanosensing in many different types of bacteria. Similarly to inhibiting flagellar rotation, inhibiting the retraction of type-IV pili upon surface contact can also result in a biological response (Fig. 4B). For instance, when type-IV pili of *P. aeruginosa* attach to a surface and start to retract, tension is generated in the pili; this tension is mechanically transferred through PilA (the major pilin subunits) that can interact directly with PilJ (a chemoreceptor-like protein of the Pil–Chp complex). The tension in the pili is then read out by the Chp chemosensory system within cells (Luo et al., 2015; Persat et al., 2015a). The Pil–Chp system subsequently increases cAMP production, which, in turn, activates virulence (Luo et al., 2015). Additional evidence comes from other work showing that type-IV pili can be co-localized with components of the Chp system to coordinate signaling leading to cAMP-dependent upregulation of virulence of *P. aeruginosa* on surface contact (Inclan et al., 2016).

Notably, the putative mechanosensor PilY1, which as discussed above is an envelope protein in *P. aeruginosa* cells, appears to also be involved in the activation of virulence that is triggered by surface-sensing. At least two groups of researchers have found that PilY1 plays a critical role in increasing the virulence of *P. aeruginosa* upon surface contact (Luo et al., 2015; Siryaporn et al., 2014). Siryaporn et al. (2014) found that surface contact cannot activate virulence in *P. aeruginosa* lacking PilY1, whereas loss of the VWFa domain from the PilY1 protein leads to hyperactivated virulence, even without any surface contact. This suggests that the VWFa domain of PilY1 may be responsible for surface detection. Both type-IV pili and PilY1 can induce virulence upon surface contact, which suggests that these two components may work inter-relatedly. This speculation is supported by other work showing that the secretion of PilY1 depends on the type-IV pili assembly system and that PilY1 signals through the type-IV pilus alignment complex to activate c-di-GMP production (Luo et al., 2015). The roles of c-di-GMP are discussed above in the section ‘Response to mechanical cues’.

In addition to changes in virulence, changes in the motility of some surface-associated bacteria and their descendants appear to be another mechanoresponse to surface sensing. Surface-attached *P. aeruginosa* can divide asymmetrically to generate a daughter cell that remains on the surface and a second daughter cell that detaches from the surface to colonize distant sites (Laventie et al., 2019; Lee et al., 2018). Both daughter cells can use intercellular cAMP levels and type-IV pili activity to provide a ‘memory’ that their ancestor cell had been attached to a surface and thereby promote stronger subsequent attachment and lower surface motility (Lee et al., 2018).

Moreover, some bacterial species may have more than one pathway to sense surfaces. For instance, *C. crescentus* appears to have two mechanosensory structures that are both involved in the same process, as follows. The holdfast is a nanoscopic adhesive produced by *C. crescentus* that helps bacteria to strongly attach to surfaces and resist displacement by flow. It was found that the holdfast adapts its elastic response from initially heterogeneous to more homogeneous with increasing time after surface attachment (Hernando-Pérez et al., 2018). In addition, it has been shown that resistance to flagellar rotation owing to surface contact in fact results in the formation of the holdfast (Hug et al., 2017). Furthermore, physically blocking the retraction of type-IV pili (and thus increasing the mechanical load on the pili during retraction) was sufficient to stimulate c-di-GMP production and initiate holdfast synthesis, even in the absence of surface contact (Ellison et al., 2017). Therefore, both inhibition of the flagellar rotary motor and inhibition of type-IV pili retraction motors are involved in surface sensing in this organism. These findings also suggest that the synthesis and development of the holdfast, like other attachment-dependent bacterial behaviors, may be regulated by mechanosensing.

There are many other examples where bacterial appendages that are driven by motors have been linked to surface sensing or to surface-associated behaviors. Although in most cases the role of the motor was not specifically probed, in combination with the evidence highlighted above, these may be taken as indirectly suggestive of mechanosensing. There is a vast primary literature on this topic and we refer the reader to recent relevant review articles for further details (Chang, 2018; Persat, 2017). In addition to the role of bacterial appendages in surface sensing, we would like to reiterate here that proteins in the bacterial envelope have also been linked to surface sensing, and as noted above, there is evidence that

these envelope proteins also have a mechanosensory role (Luo et al., 2015; Otto and Silhavy, 2002; Rodesney et al., 2017).

Responses to substrate stiffness

Much of what is known about eukaryotic mechanobiology has been identified through studies of how varying the viscoelastic properties of the substrate (i.e. substrate stiffness) affects the cell (Discher et al., 2005; Kim et al., 2009). The solid-like stiffness of a material, i.e. the resistance, or energy cost, for deforming the material and therefore the mechanical energy that can be stored in the material as a result of deformation, is measured using an elastic modulus, which can be specific to stretching, shearing or compressing the material.

The motility of *P. aeruginosa* and of *E. coli* on a surface varies with the elasticity of that surface. For *E. coli*, this depends on the flagellar rotation, which, as we have discussed above, is likely a mechanosensor for many bacteria (Song et al., 2017). *P. aeruginosa*'s response to substrate elasticity involves c-di-GMP, which as discussed above appears likely to also be a major player in mechanoresponse, and *oprF* (Song et al., 2018). A number of groups have determined different effects of substrate stiffness on bacterial adhesion, suggesting that mechanosensing and mechanoresponse may play a role in the initial bacterial 'decision' whether to remain on a surface. For instance, for *Staphylococcus aureus*, the number density of adherent cells on the surface of polyacrylamide hydrogels decreased as the modulus of the gel substrate increased (Wang et al., 2016). Here, adhesion was reduced by three logarithmic scales with increasing modulus (from 17 Pa to 654 Pa). This trend was further amplified for biofilms, with the formation of biofilms on substrates decreased as the substrate modulus increased (Wang et al., 2016). In addition, another group observed that a decrease in the elasticity of polydimethylsiloxane (PDMS) (from 2.6 MPa to 0.1 MPa) also promoted the attachment of *E. coli* and *P. aeruginosa* (Song and Ren, 2014). *E. coli* cells attached to stiff substrates can be more motile than those on soft substrates (Song et al., 2017); this difference in motility might lead to higher rates of bacterial detachment from stiff surfaces.

Findings from another group, however, appear contradictory to those discussed above. In this case, *S. aureus* and *E. coli* were allowed to attach to poly(ethylene glycol) dimethacrylate hydrogels, as compared to the studies discussed above which used PDMS gels, and were found to be more likely to adhere when the stiffness of the substrate was increased (from 44 kPa to 6489 kPa) (Kolewe et al., 2015, 2018). In this research, the thickness of the gel substrate deposited on the glass coverslip also impacted on the likelihood of bacterial adhesion – more bacteria attached on the thin gel (∼15 µm) than the thick gel (∼150 µm). The authors speculated that the very stiff underlying glass coverslip was causing the thin hydrogels to feel stiffer to the bacteria (Kolewe et al., 2018). Thus, the effective compliance of the composite material, which is made up of both the gel and the glass coverslip, is what impacts bacterial adhesion.

By what mechanism(s) bacteria respond to substrate stiffness is not known. The apparent contradictory results described above suggest that multiple factors, including the chemistry and adhesivity of the surface, may need to be disentangled from mechanical properties. For this, intensive studies about bacterial behavior on substrates that are well controlled and systemically varied in their elasticity, thickness, and surface chemistry and adhesivity are needed.

Responses to surface topography

Much research has shown that bacterial adhesion can be strongly influenced by topographies, ranging from nanoscale- to microscale-defined structures (Anselme et al., 2010; Song et al., 2015). Researchers have tested surfaces patterned with regular topographic features that are intended to prevent bacterial adhesion (and might therefore be used to create improved surfaces for application in medicine and industry), but several studies showed that the initially anti-adhesive topographies reversed and instead significantly increased bacterial adhesion over longer times (Friedlander et al., 2013; Wang et al., 2015a). This phenomenon was not due to changes of the surface energy (i.e. wetting status) of the substrate after longer exposure with bacterial culture. Rather, bacteria might in fact use their flagella to explore and eventually settle into initially unfavorable topographies (Friedlander et al., 2013; Wang et al., 2015a). Since small pits and canyons could act to restrict flagellar rotation, thus providing a mechanical signal, these may constitute additional examples of bacterial mechanosensing (Fig. 1v).

Dependence of biofilm properties on the mechanical shear imposed during growth

Many studies have shown that biofilms grown under conditions of high fluid shear are more elastic and denser in polymers and proteins than biofilms of the same bacterial strain grown under low fluid shear (Araújo et al., 2016; Fonseca and Sousa, 2007; Herbert-Guillou et al., 2001; Lemos et al., 2015; Peyton, 1996; Stoodley et al., 2001). This adaption allows biofilms to have more cell–surface and cell–cell attachment structures and to be more resistant to the detachment caused by fluid shear (Araújo et al., 2016; Lemos et al., 2015).

To our knowledge, the underlying mechanism(s) by which biofilm mechanics and composition are altered by the fluid shear conditions under which they are grown is not known. We suggest that this phenomenon may reflect the effects of mechanosensing by bacteria either early in biofilm formation and/or within the matrix of the maturing biofilm itself. This idea is consistent with other findings, discussed above, which show that the widespread second messengers c-di-GMP and cAMP could be activated by mechanosensing. Among many other things, these intracellular signals regulate the production of matrix polymers and proteins that contribute to biofilm elasticity. For example, c-di-GMP stimulates the synthesis and secretion of the alginate and Pel exopolysaccharides that can be major components of the matrices of *P. aeruginosa* biofilms (Hengge, 2009). To study the underlying links between the higher elasticity and density of biofilms when grown under high shear and the possible causative role of a higher c-di-GMP and/or cAMP production, mutants that are deficient in the generation of c-di-GMP and/or cAMP could be used to measure how they vary with shear. We hypothesize that biofilms from such mutants will not exhibit a dependence on elasticity and density with the shear applied during growth. Alternatively, c-di-GMP and/or cAMP levels in biofilms could be measured, e.g. using fluorescent reporter proteins, *in situ*, when different shears are applied.

Conclusions

Bacterial mechanosensing allows bacteria to adapt to mechanical cues from the dynamic environments in which they live; adapting to changing mechanical environments and responding to mechanical cues is likely to be of great importance to bacterial survival and evolution. In bacterial mechanosensing, active motors and envelope proteins serve as mechanosensors and trigger biological responses, with increased c-di-GMP and cAMP signaling and increased virulence being prominent examples. However, very little is known about the molecular pathways leading from mechanical inputs to biochemical signals within bacterial cells. Furthermore, as we

outline above, there is a plethora of indirect evidence for bacterial mechanosensing. Thus, bacterial mechanosensing is an emerging field with a great deal still to be further investigated. Here, interdisciplinary collaborations, including the fields of physics, chemistry, molecular microbiology and engineering are likely to be fruitful. We anticipate that an increased understanding of this aspect of bacterial cell biology will allow the development of novel approaches to manipulating bacteria, both to control unwanted infections and contamination, and to promote beneficial processes where desired.

Acknowledgements
We thank Jacob Blacutt (University of Texas, Austin, USA) for a preliminary gathering of literature on this topic.

Competing interests
The authors declare no competing or financial interests.

Funding
This work was supported by grants from the Cystic Fibrosis Foundation (GORDON17I0), the National Science Foundation (1727544; Biomechanics and Mechanobiology; Civil, Mechanical and Manufacturing Innovation), and the National Institutes of Health (1R01AI121500-01A1; National Institute of Allergy and Infectious Diseases), all to Vernita Gordon. Deposited in PMC for release after 12 months.

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