Review

A Skeptic's Guide to Bacterial Mechanosensing

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Abstract

Surface sensing in bacteria is a precursor to the colonization of biotic and abiotic surfaces, and an important cause of drug resistance and virulence. As a motile bacterium approaches and adheres to a surface from the bulk fluid, the mechanical forces that act on it change. Bacteria are able to sense these changes in the mechanical load through a process termed mechanosensing. Bacterial mechanosensing has featured prominently in recent literature as playing a key role in surface sensing. However, the changes in mechanical loads on different parts of the cell at a surface vary in magnitudes as well as in signs. This confounds the determination of a causal relationship between the activation of specific mechanosensors and surface sensing. Here, we explain how contrasting mechanical stimuli arise on a surface-adherent cell and how known mechanosensors respond to these stimuli. The evidence for mechanosensing in select bacterial species is reinterpreted, with a focus on mechanosensitive molecular motors. We conclude with proposed criteria that bacterial mechanosensors must satisfy to successfully mediate surface sensing.

Introduction

Motile bacterial species spend a small fraction of their lifetime in a planktonic or vegetative state in a liquid medium. In their natural habitat, bacteria spend a majority of their lifetime in surface-associated states, for example, in biofilms or in swarmer colonies [1–5]. The transition from the planktonic state to a surface-associated state is typically initiated when a swimming bacterium encounters and senses a surface. The mechanisms responsible for the initiation of this transition are of considerable interest given the importance of bacterial surface colonization in host invasion and infections as well as in bioremediation and microbial fuel cells [6–9]. Furthermore, bacteria in surface-associated states tend to exhibit elevated resistance to antibiotics [10–12].

The mechanical load on a bacterium changes as it approaches and adheres to a surface from the bulk fluid. When the change in mechanical load modulates protein function, thereby regulating bacterial functions, we term the process mechanosensing. Bacterial mechanosensing is likely to play a major role in surface sensing and in the initiation of intracellular signaling [13–16], analogous to mechanosensing in mammalian and plant cells [17–22]. Proteins that sense the mechanical stimuli (termed mechanosensors) may subsequently initiate a variety of downstream effects, including changes in enzymatic activity, gene expression, or metabolism. However, bacterial cells might experience varied mechanical stimuli at a surface, which can make it challenging to identify the role of specific mechanosensors in signaling. Also surface sensing may occur through mechanisms that do not necessarily involve the detection of changes in mechanical loads. The cell could sense alterations in its chemical, electrical, or thermal environments upon surface attachment and initiate intracellular signaling [23].

To correctly determine the role of mechanosensing in the transition from the planktonic state to a surface-associated state, it is critical to understand the type of mechanical load changes experienced by

a cell at a surface. This in turn depends on how the bacterium attaches to the surface—that is, the specific organelle(s) and/or portion of the cell body which mediates the surface attachment. The nature of the surface attachment determines which mechanosensors are triggered. However, an analysis of the type and magnitude of stresses that cells encounter at a surface is lacking. This may in part be responsible for why the surface sensing mechanisms and the molecular pathways involved in bacterial mechanotransduction remain obscure.

Here, we discuss prominent modes of bacterial attachments to a surface and how they lead to mechanical load changes of varying magnitudes and contrasting signs. We explore how such opposing natures of load changes pose a challenge in the analysis and in the interpretation of experimental results. We reinterpret prominent evidence for mechanosensitive signaling in select bacterial species in the context of mechanosensors. We then conclude with criteria that bacterial mechanosensors must meet to trigger mechanosensitive pathways.

Changes in Mechanical Load Following Surface Attachment

To prevent premature turning on of mechanosensitive signaling in the bulk fluid, a bacterial mechanosensor should become activated and initiate signaling primarily in response to mechanical load changes that arise during surface attachment. To do this, the cell must ignore basal mechanical loads that exist in the bulk fluid by discriminating between load changes based on their relative magnitudes. Motile planktonic cells experience viscous resistance to their motion due to the surrounding fluid. The viscous drag force on the cell is estimated by $F_{Drag} \sim 6\pi\mu a\nu$. A typical planktonic, flagellated bacterium of 1 μm characteristic size (a) experiences a drag force ~ 0.5–2 pN when swimming at 30–100 μm/s (ν) in water (viscosity $\mu \sim 10^{-3}$ Pa s). The cell body counter rotates at a speed of $\Omega \sim 10$–20 Hz due to the rotation of the flagellar filament resulting in a resistive torque on the cell body of $\tau_{Drag} \sim 8\pi\mu a^3\Omega$, which is ~1600 pN nm. Swimming bacteria in the bulk fluid may also encounter other cells or diffusing objects. The resistive torque and the viscous drag together with the mechanical forces that arise due to contact with other objects establish a baseline viscous load that is ever-present on the cell body. When attachment to a surface obstructs motility in a quiescent fluid (Fig. 1A), there is a dramatic decline in the shear load on the cell body relative to the baseline (Fig. 1B). These negative mechanical load changes occur the moment a cell ceases to swim. Thus, the cessation in motility itself causes a prominent mechanical stimulus on the cell body, which may activate putative mechanosensors.

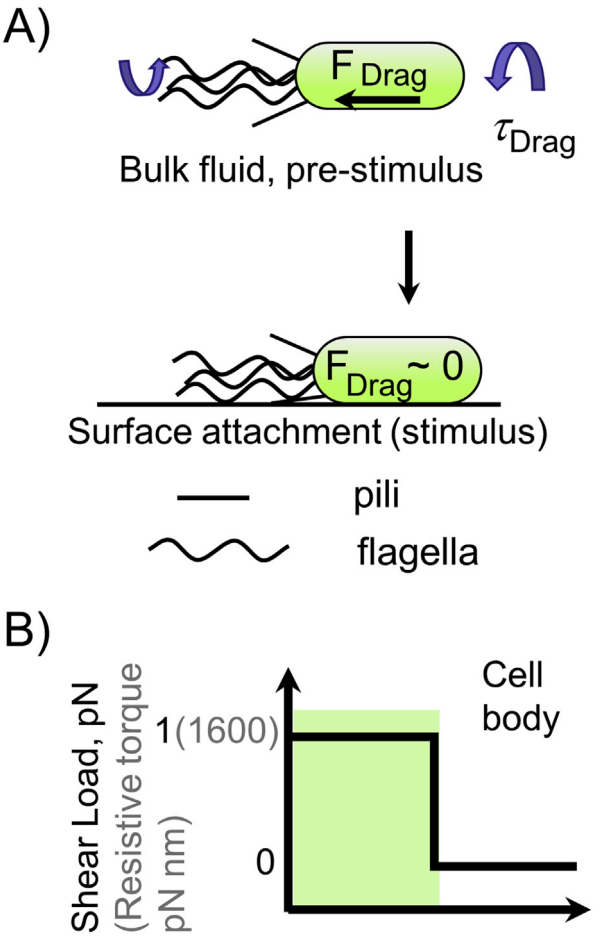

Fig. 1. (A) A swimmer experiences a persistent viscous drag force (F_{Drag}) and a resistive torque (τ_{Drag}) due to its motility (top). When it encounters a surface that obstructs its motility, it immediately experiences a reduction in F_{Drag} and τ_{Drag} in a quiescent fluid. (B) The prestimulus load on the cell body is proportional to the swimming speed and the cell's counter-rotation frequency. Attachment to a surface causes a negative change in load: $\Delta F_{Drag} \sim -1$ pN, and $\Delta\tau_{Drag} \sim -1600$ pN nm.

A membrane-embedded motor that actuates an extracellular appendage is a natural candidate for mechanosensors. This is because the motor can track changes in the mechanical loads by sensing changes in the viscous resistance to the movement of its appendage (i.e., the viscous load). Two motor-driven appendages, the flagellum and the type IV pilus, have been implicated in surface sensing [24,25]. The flagellum consists of an extracellular flagellar filament that is rotated by an electric transmembrane motor. The flagellar motor itself consists of a multiunit stator that generates torque to rotate the rotor with the aid of the proton (ion)-motive-force [26]. The type IV pilus consists of an extracellular filament that extends and retracts due to the action of ATPases. For the flagellar and pilus

motors to sense surface attachment and initiate signaling via mechanosensitive mechanisms, the viscous load on the respective appendages must change following the attachment. No mechanosensors reportedly exist within the extracellular filaments themselves. Hence, the specific nature of the filament interactions with the surface is unlikely to be relevant.

The magnitudes of load changes on these appendages depend on the manner of cell attachment to the surface (Fig. 2A). For example, the cell body might attach to the surface such that the appendages remain free (scenario I). Alternatively, the cell body including some or all the appendages may adhere to the surface (scenario II). Often, a single flagellum alone may attach to the surface (Fig. 2B). This is typically achieved in the laboratory with the aid of flagellin variants that stick readily to hydrophobic surfaces [27], or with antiflagellin antibodies that link the flagellum to the surface [28,29]. In these cases, the cell has been "tethered" and it rotates. Rotation of the cell body does not require flagellar tethering to a surface though. It can also occur because of hydrodynamic interactions of the rotating flagellum with the surface (Fig. 2C [30]).

The load on the flagellar motor due to viscous drag on the rotating flagellar filament in a swimming cell is ~5 pN nm s/revolution. The motor experiences a significant mechanical load (>150 pN nm s/revolution [31]) if the filament adheres to the surface (Fig. 2A, scenario II and Fig. 2B). As a result, the attachment corresponds to a significant increase in the load on the flagellum of >145 pN nm s/revolution

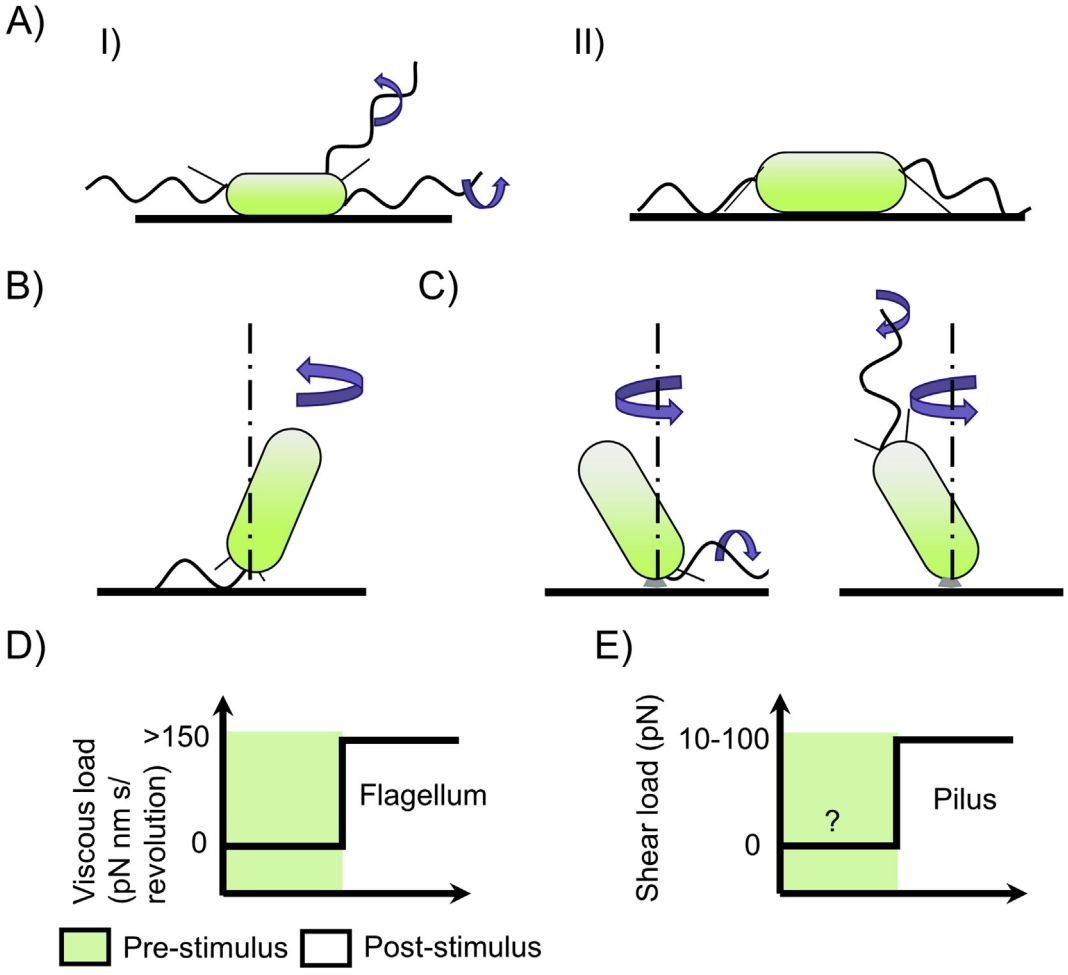

Fig. 2. (A) Scenario I—cell body is surface-attached but the appendages are free and unloaded. Scenario II—the cell and prominent appendages become surface-attached and thus, loaded. (B) Flagellar attachment to the surface will cause a rotation of the cell provided other appendages remain unattached. (C) Cases where cell may attach to a surface due to the presence of adhesive components on its body, and is able to freely pivot around the joint. Cell rotation occurs either due to hydrodynamic interactions of the rotating flagellum with the surface (left) or due to off-axis flagellar thrust that generates a torque on the cell (right). Another possibility is that the cell simply counter rotates around the fluid joint due to on-axis flagellar rotation. (D) and (E) Increase in flagellar and pili-loads when the respective appendages attach to the surface and are stalled, as indicated in scenario II.

(Fig. 2D). In contrast, the flagellar load changes are modest in a rotating cell body that appears to be tethered via its flagellum, but is actually surface-adherent because of a fluid joint (Fig. 2C). Thus, flagellar-mediated mechanosensitive signaling is more likely if the filament attaches to the surface (Fig. 2A and B). It is unlikely to occur if the cell body, rather than the filament, is attached to the surface (Fig. 2C).

The pili are free from any attachments in a swimming cell. Therefore, they should experience negligible tensile loads during swimming. When they adhere to the surface, they are capable of pulling and moving the entire cell body, resulting in twitching motility [32,33]. Tensile forces within the extracellular filament balance the retracting force applied by the force-generating enzymes in the pilus, and these forces can range from 8 to 100 pN [34–37]. The tensile forces act as the load on the force-generating pilus motor. The load is high when the pilus adheres to the surface (e.g., scenario II, Fig. 2A), and it is negligible when the pilus detaches from the surface and extends (e.g., scenario I, Fig. 2A). Because the pili stochastically attach and detach from the surface [38], the load on the pilus motor changes over time. If the time-averaged load change experienced by a surface-adherent pilus is high (Fig. 2E), it is likely to promote pili-mediated mechanosensitive signaling. If the pili merely extend and retract without physically interacting with the surface, no change in load occurs. In such a case, downstream signaling is not likely to be pili-mediated.

In each of the aforementioned representative scenarios the type and magnitude of mechanical load change is different. Therefore, the knowledge of the nature of surface attachment is crucial to discriminate between mechanosensitive and non-mechanosensitive signaling during bacterial-surface interactions. However, the appendages are not easily observable in standard microscopy experiments, and it is not straightforward to determine how they interact with the surface [39].

In addition to the changes in load depicted in Figs. 1 and 2, the bacterium may experience other types of mechanical stimuli at a surface, especially when exposed to fluid flows. Gravitational forces are negligible because of the small size of the bacterium. Likewise, the reaction force from the surface that balances the gravitational pull is also negligible. But strong adhesion interactions can occur between the surface and the cell [23]. These forces can resist detachment forces as high as a few nN [40] and are predicted to cause cell wall deformations [41]. Mechanosensitive proteins within the cell membranes are believed to be able to detect deformations in the cell wall [15,42]. However, significant cell deformations do not seem to occur in surface-adherent wild-type bacteria even over an hour following attachment [43].

As should be evident from the foregoing discussion, not only the magnitudes but also the signs of the surface-induced mechanical stimuli are different on different parts of the cell. Several types of load changes can occur simultaneously during surface attachment. For example, when a swimming bacterium adheres to a surface along with its appendages (Fig. 2A scenario II), there is a negative load change on the cell body due to the reduction in viscous drag with a simultaneous positive load change on the flagellar and the pili motors. There may be an additional positive load change in the form of increased adhesive forces on the attached cell in the presence of hydrodynamic flows. This suggests that a primary criterion for the effective functioning of a bacterial mechanosensor is that it must be able to discriminate between the magnitudes as well as the signs of mechanical stimuli.

In the next sections, we discuss the response of bacterial mechanosensors to load changes and the challenges in interpretation of experimental results involving bacterial mechanosensing.

Sensing of Load Changes

The levels of select messenger molecules in the bacterium can change when the cell attaches to a surface, for example, the global regulators cyclic diguanylate monophosphate (c-di-GMP) and cyclic adenosine monophosphate (cAMP). In several bacterial species, these messengers regulate a variety of cellular processes such as surface colonization, biofilm formation, cell cycle regulation, and virulence [14,44–49]. Here, we focus on how mechanosensors, especially mechanosensitive molecular motors, detect load changes to modulate the levels of regulatory molecules.

Mechanosensing by motors

Mechanosensing by the flagellar motors: Flagella have been implicated in sensing of and cellular adaptation to surfaces in numerous bacterial species [2,25,50,51], and large number of bacterial species carry flagellar genes [52]. To determine how flagella respond to mechanical load changes (Fig. 2D), Lele and coworkers used optical traps to stick beads to shortened flagellar filaments in *Escherichia coli* cells. Because the load on the flagellar motor scales cubically with the size of the object that it rotates, attachment of the bead to the short flagellum instantaneously increased the load on the motor by a factor of ~8000 [53]. In an alternate experiment, they tethered the cell to a surface similar to the manner depicted in Fig. 2B. In either case, the flagellar stator complex responded by adding ~6–11 stator units to increase the flagellar power under high loads. The remodeling was observed in strains

lacking FliL, which had been suggested to mediate flagellar mechanosensing. Remodeling also occurred in strains lacking the protein that forms the extracellular filament (FliC) as well as those in which the motor rotation was locked in the clockwise or counterclockwise direction. These and other works suggested that the flagellar stator itself is likely the mechanosensitive protein complex in the motor [31,53,54]. Another study showed that such load-dependent binding of stator units to individual motors was persistent; the remodeled units continued to associate with the motor despite the stalling of rotation for several minutes [55]. The structural remodeling of the stator complex and the resultant functional adaptation in response to flagellar load changes have so far been reproducibly observed in *E. coli* and *Bacillus subtilis* [31,53,55–59].

The viscous load on the extracellular flagellum should be experienced by the flagellar motor only so long as the stator continues to generate torque. Consistent with this idea, torque-generating stators were observed to remodel when the flagella were tethered to the surface, but paralyzed stators, which were unable to generate torque, did not remodel in tethered cells [31]. These findings are consistent with a model in which the unbinding/binding rates of a stator unit to the motor are controlled by the torque the unit generates [31]. There is additional support for this idea; recent experiments indicate that the unbinding rates of stator molecules decrease as torque increases, and the on-rates decrease with speed when the motor speeds are high [60].

Mechanosensing by stators may contribute to the initiation of biofilm formation, swarming, increased expression of virulence genes, as well as in the regulation of genetic competence [50,61]. The mechanisms are unknown. One possibility is that stator remodeling under high loads modifies the local cell membrane potential, which could subsequently initiate signaling [62]. Or the increased torque upon remodeling might modify rotor interfaces to enhance the binding of downstream effectors that are involved in signaling. Another model involves the depletion of the pool of free stator units in the cell due to load-dependent remodeling. The depletion in free stator units could trigger downstream signaling. There is some experimental support for this. For example, two types of stators, MotA-B and MotC-D, are responsible for flagellar rotation in *Pseudomonas aeruginosa*. MotC-D is recruited by the motor in preference to MotA-B under high loads, which appears to modulate interactions with diguanylate cyclases and the levels of c-di-GMP [47,61]. However, there are around 100–200 total stator units in a cell [63], with each motor likely binding around 4–6 units at loads experienced in swimming cells, and no more than 11–16 units at maximum loads [64–66]. In the depletion model, the messenger molecule levels would have to be very sensitive to small changes in the number of freely available stator units in the cell (of the order of 7–10 stator units). In species such as *P. aeruginosa* which carry a lone flagellar motor, the reduction in the number of free stator units upon surface attachment is expected to be <5%. This mechanism poses a challenge as it may necessitate an impracticably tight control over cell-to-cell variability in stator protein copies.

Mechanosensing by the type IV pilus: Mechanical stimuli–induced structural modifications and functional adaptations that are readily measurable in flagellar motors have not been reported in the type IV pilus yet. However, the activity of the motor enzymes is likely responsive to mechanical contact between the tip of the pilus and a surface [67]. Sensing of the surface by the pilus is typically inferred from subsequent downstream effects in signaling [68–73]. In *P. aeruginosa*, the extracellular pilin filament consists of PilA subunits, and the retraction is facilitated by the ATPase PilT. PilT has been implicated in downstream signaling events and the upregulation of virulence [70,74]. If PilT or other enzymes are capable of sensing the tensile load in the extracellular filament, they could function as mechanosensitive proteins similar to stator proteins in the flagellar motor.

A prominent example of pili-mediated posttranslational regulation is that of holdfast induction in *Caulobacter crescentus* [73]. The holdfast is a strong adhesin which irreversibly attaches a cell via its pole to a surface, resulting in rapid surface colonization. The induction begins almost immediately following surface attachment [75,76]. Although some evidence indicates that the pili induce holdfast synthesis, other experimental observations point to a prominent role for the flagellar motor instead [77]. This might likely be due to a cross-talk between the two appendages [37,78]. Interestingly, holdfast induction is observed even in strains lacking the pili and the extracellular components of the flagella, so long as functional components of the flagellar rotor and the stator units were present within the cell body [77]. This type of sensing has been termed as tetherless surface sensing. As discussed before, without the extracellular tethers, motors are unlikely to sense changes in extracellular viscous loads. Hence, whether surface sensing by the pili and the flagella in *C. crescentus* is evidence of mechanosensing remains an open question.

Mechanosensing with nonmotor proteins

Putative nonmotor bacterial mechanosensors include protein sensors that may reside on the cell surface. Motile cells in gram-negative species may sense reduction in the viscous drag on their bodies (Fig. 1B) with the aid of outer-membrane mechanosensors. Alternately, the sensors might undergo conformational changes due to the proximity to

charged entities on a surface or due to some other reason [23]. Parsing the extent to which these sensors respond to surface conditions versus changes in mechanical load is a significant challenge.

Candidate mechanosensors include the outer-membrane lipoprotein NlpE in *E. coli* which likely triggers the CpxA-R two-component signaling pathway upon surface contact [79]. The CpxA-R system is involved in the invasion of host cells as well as in multidrug resistance [80–83]. Recent work implicates the RcsCDB phosphorelay system in surface sensing, which involves the outer-membrane lipoprotein RcsF [84]. The Rcs system regulates biofilm growth, modulates the expression of motility genes, and mediates a variety of bacterial functions [85]. In *P. aeruginosa*, outer-membrane protein PilY1 likely mediates bacterial attachment to surfaces [71,86]. PilY1 associates with the pilus and is essential for its biogenesis [87]. Therefore, PilY1 is essential for the mechanosensitive function of the pili even though it is not involved in force generation.

Outer-membrane mechanosensors must meet several criteria to function properly. A thin fluid layer ~ 5–20 nm separates the cell body and the surface [88,89]. Putative mechanosensors must be able to span this distance to interact with and sense the surface. Any part of the bacterial outer membrane may randomly come to rest on the surface, and the area of contact between the bacterial body and the surface is typically small (Fig. 3A). Therefore, any putative membrane-embedded mechanosensors have to be distributed in large numbers throughout the bacterial surface.

How might membrane mechanosensors distinguish between attachment to a surface and the occasional contact with objects in the bulk fluid? One possibility is that the cell relies on temporal integration of the signal that is received from the mechanosensors such that once a signal threshold is exceeded, mechanosensitive pathways are triggered. In such a mechanism, transient contacts will be filtered out as the integrated signal will remain below the threshold. If, however, the cell relies on spatial integration of sensor signals, then a high sensitivity to mechanical contact and a high degree of cooperativity among the contacting and noncontacting sensors could help limit the number of sensors required on the cell body, while possibly resolving conflicting signals. A good example of high cooperativity is seen in bacterial chemoreceptors that are distributed in dense patches in the inner membrane [90,91].

Ion channels such as MscL and MscC in the inner membrane of bacteria respond to stresses generated in the membranes [41,92,93]. In addition to

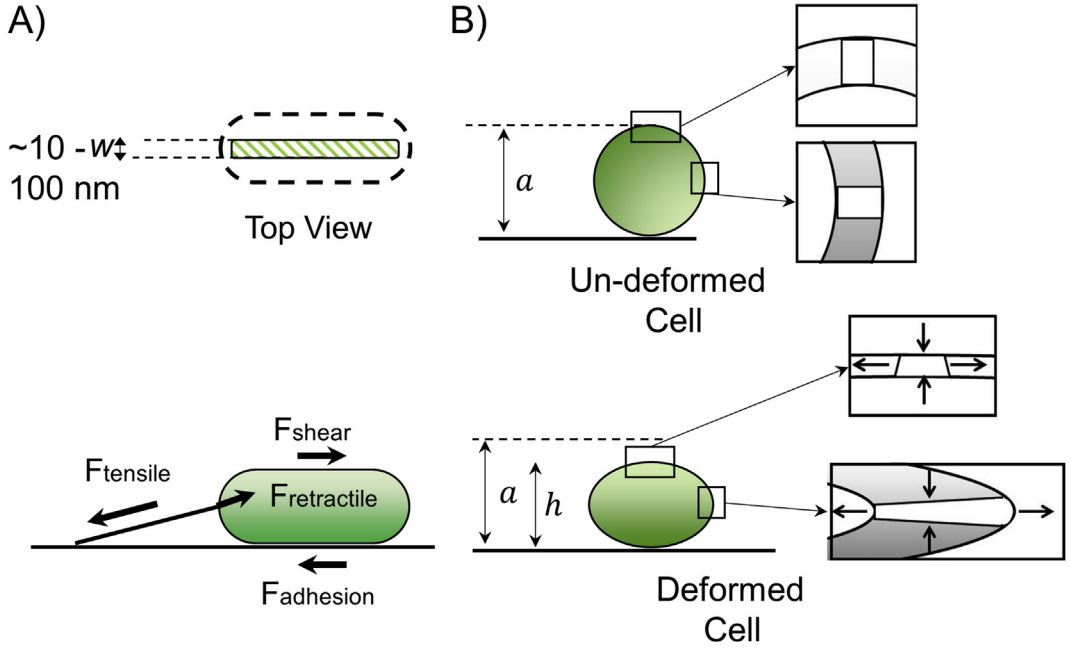

Fig. 3. (A) *Top*: The contact area between the surface and the attached cell is a tiny fraction of the overall surface area of the cell (top view). The adhesion forces act only on this area. *Bottom*: In a nonquiescent fluid, the tensile force on a surface-adherent pilus is a combination of the shear force, the adhesion force, and the retractile force. When the fluid flow is in a direction opposite to the retractile force, it reduces the total tensile force. (B) An attached cell viewed along its pole. *Top*: the undeformed cell diameter is *a*. Inactive putative mechanosensitive channels embedded within the cell membrane are indicated in the inset figures. *Bottom*: The cell deforms over a period of time to *h*, and the channels along the top and side sections of the cell experience contrasting forces as indicated by the arrows in the inset.

adhesion forces, the strong forces generated by the pilus and/or flagellar motors upon surface attachment could modulate the mechanical stresses within the membranes and trigger these sensors. Typical host surfaces such as cells, tissues, or mucosal layers are soft and deformable. Their flexibility will limit the development of mechanical stresses in bacteria that colonize them. The cell wall is highly rigid (~0.1 GPa [94–96]) owing in part because of the turgor pressure [97]. For a characteristic cell dimension of ~1 μm, a force of 100 pN motor force will cause insignificant overall cell wall deformation (<1 nm). If significant deformation does occur, different parts of the cell wall will experience either compressive or tensile stresses, resulting in contrasting stimuli on putative sensors (Fig. 3B). Much work remains to be carried out to uncover whether and how nonmotor mechanosensors respond to such contrasting and small mechanical stresses.

Dynamics of Mechanosensing

The bacterium likely discriminates between real mechanical signals that arise due to surface attachment from those that might be transient or short-lived, such as the occasional interactions with another cell or solid objects in the bulk fluid, by sensing the persistence of mechanical signals. Sensing only those stimuli that persist for long enough times may help reduce the occurrence of erroneous signaling. Measurements of the relevant time scales are limited, but they are likely to be on the order of several seconds. For example, in *E. coli*, stator remodeling initiates within ~10 s after the load change on the flagellar motor [53,98], whereas in *P. aeruginosa*, the flagellar motor modulates c-di-GMP levels within a few seconds of surface attachment [99].

For a motor-based mechanosensor, temporal persistence in mechanical stimulus is force- or torque-dependent as inhibition of force/torque dissipates the load. For nonmotor-based mechanosensors, temporal persistence in mechanical stimulus is probably dependent on the specificity of adhesive interactions with surfaces. For example, the type 1 fimbrial FimH adhesin binds with high specificity to D-mannose, and the adhesion is shear-dependent [100–102]. Specificity in binding might help trigger specific pathways due to strong and persistent interactions. Nonspecific adhesions are likely to be transient and weak, therefore such interactions may not trigger specific signaling pathways, or may be too slow to initiate signaling. Testing of these ideas will likely contribute to a better understanding of mechanosensing.

Disruption in enzymatic functions via genetic modifications has been widely used to study the role of mechanosensitive proteins in the initiation of intracellular signaling. However, bacterial mechanosensing and mechanotransduction are dynamic processes, and approaches that involve load changes through genetic modifications may not be optimally suited for exploring these dynamics. This is because the mechanosensitive response may adapt to the loss or mutation of a putative critical enzyme. If the responses before and after genetic modification happen to be similar, then the role of the mechanosensitive protein encoded by that gene may remain undetected in experiments altogether.

Mechanosensing, Shear Rate Sensing, and Swimming-Speed Sensing

A common approach to mechanically stimulate bacteria is to attach them to a surface and then apply increasing shear stresses with fluid flows (Fig. 3A). Recent work indicates that c-di-GMP levels in surface-adherent *P. aeruginosa* increase with flow rates, with current interpretation being that the hydrodynamic shear stresses are responsible for the increase [86]. However, the maximum viscous stresses (F_{shear}) on the bacterium in such experiments are typically limited to ~ 0.01 pN [86], partly owing to the small chamber sizes in laboratory flow cells. These viscous stresses are tiny relative to those experienced by swimmers (F_{Drag} ~ 1 pN, Fig. 1B). If high shear stresses elevated c-di-GMP levels, then the levels would be highest in swimming cells, which is not the case. The loads on the pili with or without the hydrodynamic flows are also similar as the tensile loads on a surface-adherent pilus are already ~100 pN (Fig. 2E). An F_{shear} ~ 0.01 pN or less is not expected to change pilin loads appreciably (Fig. 3A). Hence, it is unlikely that the pili respond mechanosensitively to the applied shear stresses. It is possible that mechanosensing is not involved in the regulation of c-di-GMP due to fluid flows.

Hydrodynamic flows reduce the concentrations of secreted chemical species that build up near surface-adherent cells. The cell might sense the depletion of these chemicals rather than the shear stress to initiate intracellular signaling. This notion is consistent with recent findings that suggest that the expression of certain genes in *P. aeruginosa* is regulated by hydrodynamic shear rates rather than the shear forces [103]. Because the shear rate in such experiments is a measure of the fluid velocity relative to the surface-adherent cell body, another way of interpreting the results is to ask whether the expression of genes is tied to the swimming speed of the bacterium in quiescent fluid. Higher the swimming speed of the cell, greater the apparent shear rate. An intriguing possibility then exists—does the cell sense its swimming speed to regulate signaling? One mechanism for the cell to determine its speed is to keep track of the flagellar rotation rates based on

the proton (or ion) influx through the stators. A different mechanism could be to track the levels of endogenously produced and secreted chemical species, possibly with the aid of the chemotaxis network.

Not all mechanosensitive signaling pathways are surface-dependent. For example, the transition of *B. subtilis* into the so-called K-state promotes natural competence [104]. Entry into the K-state is independent of surface adhesion and correlates with basal levels of DegU phosphorylation. DegU is a transcriptional regulatory protein and a response regulator in *B. subtilis* that regulates genetic competence and biofilm formation [105]. Diethmaier and coworkers recently observed that a reduction in flagellar loads correlated with elevated DegU-P levels and reduced K-state transition probabilities [106]. Flagellar stalling also seems to elevate DegU-P levels [51,107]. This surface-independent, flagellar-mediated control of DegU-P levels appears consistent with a mechanism in which the viscous load on the flagellum is optimal in a wild-type swimmer for maintaining basal DegU-P levels. As flagellar mechanosensing is mediated by flagellar stators, it is possible that these proteins regulate DegU-P. Yet, in the aforementioned experiments motility was disrupted due to the interference with flagellar functions. Therefore, it is possible that the reduction in the viscous drag (Fig. 1B) activated mechanosensors on the cell surface other than the flagellum. Another possibility is that a loss of motility may by itself have activated signaling through unknown mechanisms.

Summary and Future Directions

In summary, mechanosensing can help a bacterium sense a surface provided there is a change in the mechanical load. The viscous drag on a motile cell is significant (~1 pN) and is higher than the shear stresses that can be applied on the cell in most flow cell experiments. A loss in motility causes a dramatic reduction in the viscous drag which itself can trigger mechanosensors on the cell. The two prominent mechanosensitive appendages, the pilus and the flagellum, will experience viscous load changes when attached to a surface provided their respective motors continue to generate a force or a torque. The ability to sense differences in the signs and magnitudes of the load change, the ability to detect temporal persistence in mechanical stimuli, and a high sensitivity to mechanical signals are key attributes of effective bacterial mechanosensors.

There are multiple challenges that limit our understanding of bacterial mechanosensing. First, the dynamics of mechanosensing are relatively understudied and not well understood. Challenges also exist in the design of experiments for stimulating putative mechanosensors. Among these is determining whether a particular appendage such as the flagellum or the pilus is actually loaded in a mechanical stimulation assay. To obtain unambiguous evidence regarding the role of a specific type of appendage in mechanosensing, it may be necessary to simultaneously load all the appendages of that type on the cell. This can be challenging, in part, because of the technical difficulties in appendage visualization. Exciting new techniques based on interferometric approaches [67,108] are addressing some of these challenges. To distinguish between signaling events that are activated by mechanosensing from those that may be activated by other types of surface-sensing phenomena, a comparative analysis of the mechanosensing response in the bulk fluid away from any surfaces will be necessary. Determining how mechanosensors initiate posttranslational modifications and how various regulatory events are controlled by shear rates on the cell are among the key questions for the future.

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