Type IV pili mechanochemically regulate virulence factors in *Pseudomonas aeruginosa*

Alexandre Persat[a], Yuki F. Inclan[b,c], Joanne N. Engel[b,c,d], Howard A. Stone[e], and Zemer Gitai[a,1]

[a]Department of Molecular Biology, Princeton University, Princeton, NJ 08544; [b]Department of Medicine, University of California, San Francisco, CA 94143; [c]Microbial Pathogenesis and Host Defense Program, University of California, San Francisco, CA 94143; [d]Department of Microbiology and Immunology, University of California, San Francisco, CA 94143; and [e]Department of Mechanical and Aerospace Engineering, Princeton University, Princeton, NJ 08544

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Bacteria have evolved a wide range of sensing systems to appropriately respond to environmental signals. Here we demonstrate that the opportunistic pathogen *Pseudomonas aeruginosa* detects contact with surfaces on short timescales using the mechanical activity of its type IV pili, a major surface adhesin. This signal transduction mechanism requires attachment of type IV pili to a solid surface, followed by pilus retraction and signal transduction through the Chp chemosensory system, a chemotaxis-like sensory system that regulates cAMP production and transcription of hundreds of genes, including key virulence factors. Like other chemotaxis pathways, pili-mediated surface sensing results in a transient response amplified by a positive feedback that increases type IV pili activity, thereby promoting long-term surface attachment that can stimulate additional virulence and biofilm-inducing pathways. The methyl-accepting chemotaxis protein-like chemosensor PilJ directly interacts with the major pilin subunit PilA. Our results thus support a mechanochemical model where a chemosensory system measures the mechanically induced conformational changes in stretched type IV pili. These findings demonstrate that *P. aeruginosa* not only uses type IV pili for surface-specific twitching motility, but also as a sensor regulating surface-induced gene expression and pathogenicity.

surface sensing | mechanotransduction | type IV pili | virulence

Numerous bacterial species exhibit a range of behaviors that are specific to life on surfaces. For many pathogens, surface-specific phenotypes are often associated with virulent activity, because many infection strategies require contact with a host (1). For example, in *Pseudomonas aeruginosa*, the type III secretion system, which infects by injecting toxins, requires attachment of individual cells to host-cell membranes (2). The genetic pathways that regulate *P. aeruginosa* secretion systems have been well characterized (3), but the environmental signals that activate these pathways remain poorly defined (4). Given the requirement for host-cell contact for efficient infection, *P. aeruginosa* could leverage surface contact as a signal to properly activate and coordinate pathogenicity. Indeed, our laboratory recently demonstrated that prolonged association with a solid surface contributes to *P. aeruginosa* pathogenicity when bacteria and host cells are forced together (5). Here we address the fundamental question of how bacteria rapidly sense surface contact and transduce this input into a cellular response over short timescales.

Surface-specific behaviors require intimate contact between cells and substrate. In *P. aeruginosa*, contact is mediated by several adhesins, particularly type IV pili (TFP). TFP are long, motorized fimbriae that also provide cells with surface-specific twitching motility and are essential to virulence and biofilm formation (6, 7). Successive TFP extension, attachment, and retraction promote intimate association with surfaces and motility along them. Because TFP dynamically interact with the substrate, they mechanically couple cells with surfaces. Consequently, although TFP have been viewed as adhesion and motility structures, TFP could also potentially function as mechanical sensors to rapidly signal surface contact.

The Chp system, a complex two-component signal transduction system that resembles the flagellar chemotaxis system, has previously been implicated as a regulator of TFP; in the absence of Chp proteins, *P. aeruginosa* exhibits defective TFP assembly and twitching motility, although pilin subunits are still produced (8). In addition, the Chp system has been shown to regulate the level of the signaling molecule cAMP (9, 10), which in turn binds to the transcription factor Vfr (virulence factor regulator) to activate the transcription of more than 100 genes (11). Vfr regulates multiple virulence factors in *P. aeruginosa*, including the type II and III secretion systems and quorum sensing (11). Because these virulence pathways have a high metabolic cost, such complex regulatory networks are typically optimized to sense and respond to specific environments, thereby inducing virulence in the presence of a host. The homology between the Chp system and other well-characterized chemotaxis systems (e.g., the Che system) suggested that Chp stimulates twitching upon sensing specific solutes present in the environment. However, no compound has been shown to clearly activate the Chp system (12).

Here we investigate the hypothesis that the Chp system is activated by mechanical cues transduced by TFP upon surface contact. It has been previously shown that the cAMP-dependent operon *PaQa* is up-regulated in colonies growing on solid medium (13). We show that the *PaQa* operon is directly regulated by contact between the cell and the substratum, independently of surface chemistry. We also demonstrate that the Chp chemosensory system and TFP are regulators of this mechanically induced response. We show that *P. aeruginosa* up-regulates *PaQa* in response to TFP extension, attachment, and retraction, thus identifying TFP as a mechanotransduction system. Finally, we

Significance

In their natural environments, bacteria frequently transition from a free-swimming state to a surface-associated state, attached to a substratum. As they encounter a surface, they may initiate developmental programs to optimally colonize this new environment and induce pathways such as virulence. Here we demonstrate that the pathogen *Pseudomonas aeruginosa* uses fiber-like motorized appendages called type IV pili to sense initial contact with surfaces. This leads to a signaling cascade that results in the expression of hundreds of genes associated with pathogenicity and surface-specific twitching motility. Thus, bacteria use pili not only to attach and move, but also to sense mechanical features of their environment and regulate cellular processes of surface-associated lifestyles.

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[1]To whom correspondence should be addressed. Email: zgitai@princeton.edu.

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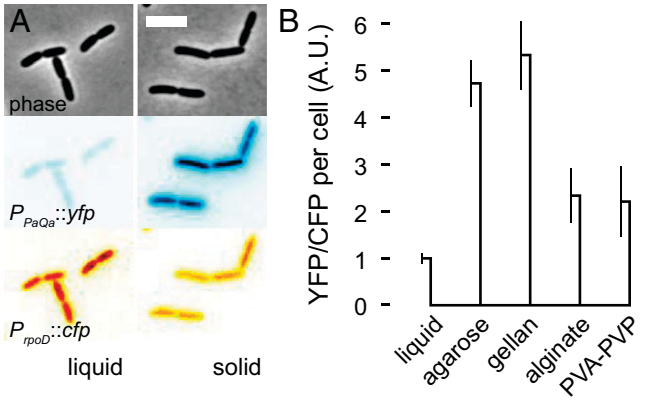

Fig. 1. Surface contact regulates the *PaQa* operon. *P. aeruginosa* was transformed with a plasmid encoding a *yfp* reporter fused to the promoter sequence of *PaQa*. A *cfp* reporter fused to the *rpoD* promoter served as an internal control. (*A*) Cells growing on solid agarose (*Right*) have higher YFP fluorescence compared with cells growing in liquid medium (*Left*), whereas CFP fluorescence only slightly decreased. (Scale bar, 1 μm.) (*B*) The fluorescence level of cell populations grown on various hydrogels for 3 h was enhanced relative to liquid growth. All fluorescence values are normalized to the levels measured in liquid. Error bars represent 95% confidence intervals ($n = 4$, each measurement is the average from more than 100 cells).

provide evidence that the major pilin subunit PilA interacts with PilJ, the sensory protein component of the Chp chemosensory system, suggesting that Chp senses TFP structural changes upon tensile load. Altogether, these findings highlight a previously unknown function for TFP in sensing the mechanical properties of surfaces upon the initial stages of surface association. TFP in turn signal through the Chp system to induce cAMP-dependent processes such as stronger adhesion, prolonged surface association, biofilm formation, and pathogenicity.

Results

***PaQa* Is Up-Regulated upon Contact with Surfaces.** To explore the role of surface contact in regulating *PaQa*, we generated a *yfp* transcriptional reporter for *PaQa* and measured its activation at the single-cell level as a ratio to a constitutively expressed *cfp* reporter. We found that single cells induced significant *PaQa* expression within 3 h of contact with a solid agarose surface (Fig. 1*A*). We observed a fivefold increase in the mean fluorescence intensity of a population of cells (Fig. 1*B*). We verified that the increase in the YFP-to-CFP ratio was mainly a result of the increased activity of the *PaQa* promoter as opposed to changes in the CFP normalization control. Specifically, we compared the distributions of CFP intensity per cell in liquid-grown and surface-associated cells; CFP distributions and their corresponding means only varied slightly between these two conditions (Fig. S1; the mean CFP fluorescence is about 10% higher in liquid than on solid media). Because both growth conditions, in liquid and on solid, were performed in the same medium, this result suggests that cells respond directly to physical contact with the agarose rather than to differences in chemical composition.

To further test this hypothesis, we characterized the response of the fluorescent *PaQa* reporter when bacteria were grown on hydrogels with distinct chemistries (i.e., harboring a variety of chemical moieties at their surface). We found that the *PaQa* reporter was induced upon growth on all hydrogels tested compared with induction in liquid, including agarose, gellan, alginate, and polyvinylalcohol-polyvinylpyrrolidone (PVA-PVP) hydrogels (Fig. 1*B*) (14, 15). We conclude that *PaQa* expression is stimulated by physical properties of surfaces rather than their chemistry, because these hydrogels harbor distinct chemical moieties.

In these experiments, cells were forced to interact with the hydrogel surface as the cell suspension was allowed to dry onto the hydrogel. However, in natural environments such as the mucosal epithelia of their hosts, bacteria would freely transition from swimming (planktonic) to surface-attached states (16). In an effort to establish the importance of surface mechanosensing in a more realistic environment, we measured the activity of the *PaQa* reporter in the presence of mucin, a major component of mucus, in a semiinfinite liquid environment where cells are free to attach or swim away from the surface. Time-lapse movies showed that single *P. aeruginosa* cells initially moved about the glass surface before developing into small colonies (Movie S1). As cells aggregated and formed larger colonies, we observed a significant increase in the activity of the *PaQa* reporter (Fig. S2*A*). As a control, we separately tested whether the use of mucin had an effect on surface sensing dynamics. We found no difference in *PaQa* induction between growth on agarose gels supplemented with mucin compared with tryptone-based lysogeny broth (LB) medium (Fig. S2*B*). These results demonstrate that the transcriptional response to surface contact occurs in an environment that mimics infections. Additionally, this shows that *PaQa* is similarly regulated upon contact with glass, a nonporous abiotic substrate.

Surface Contact Regulates cAMP Levels. Because *PaQa* transcription is dependent on cAMP levels, and cAMP levels are regulated by the Chp system (9, 17), we tested whether the mechanosensitive induction of the *PaQa* reporter depends on cAMP signaling. We repeated the surface-sensing assay on agarose using isogenic mutants defective in cAMP production or in the Chp chemosensory regulon. In *P. aeruginosa*, the *cyaB* gene encodes the major adenylate cyclase (11). In the *cyaB* mutant, the *PaQa* reporter showed no increase in fluorescence upon surface contact (Fig. S3*A*). Conversely, disruption of the gene encoding the cAMP phosphodiesterase *cpdA*, which results in elevated cAMP levels, showed constitutive induction of the *PaQa* reporter in liquid medium, without the need for surface contact (Fig. S3*B*). Finally, we verified that the surface-dependent induction of *PaQa* requires *vfr*, a transcription factor that regulates multiple virulence factors upon binding cAMP (Fig. S3*A*). Together, these results demonstrate that surface-induced *PaQa* expression requires an intact cAMP signaling pathway, indicating that surface contact is a signal for cAMP production and Vfr transcription.

The Chp Chemosensory System Regulates *PaQa* upon Surface Contact. Given the importance of cAMP, we investigated the role of the Chp chemosensory pathway, a known regulator of cAMP production and surface-associated behaviors such as twitching motility and TFP assembly (10, 12). The induction of *PaQa* upon surface contact was inhibited in mutants in the Chp chemosensory system, including mutants in *pilJ*, a methyl-accepting chemotaxis protein (MCP)-like chemosensory receptor, and *chpA*, the CheA two-component signaling histidine kinase homolog (Fig. 2*A*). Classic chemotaxis systems use CheY as the response regulator downstream of CheA (18). The Chp system has two apparent CheY homologs, PilG and PilH, which have opposite effects on cAMP levels (9). The *pilH* mutant exhibits elevated cAMP levels and, as predicted, demonstrated constitutive induction of *PaQa* expression in liquid (Fig. S3*C*). Together these results indicate that the Chp and cAMP pathways mediate the response to mechanical stimulation by surface contact. Because the Chp system is homologous to a chemotaxis system, this raises the possibility that Chp senses a signal specifically induced upon surface contact to regulate cAMP production.

Surface Sensing Requires TFP and Its Motors. How might the Chp chemosensory system sense physical contact with the surface? TFP mechanically couple the cell with the substrate. Mutants in

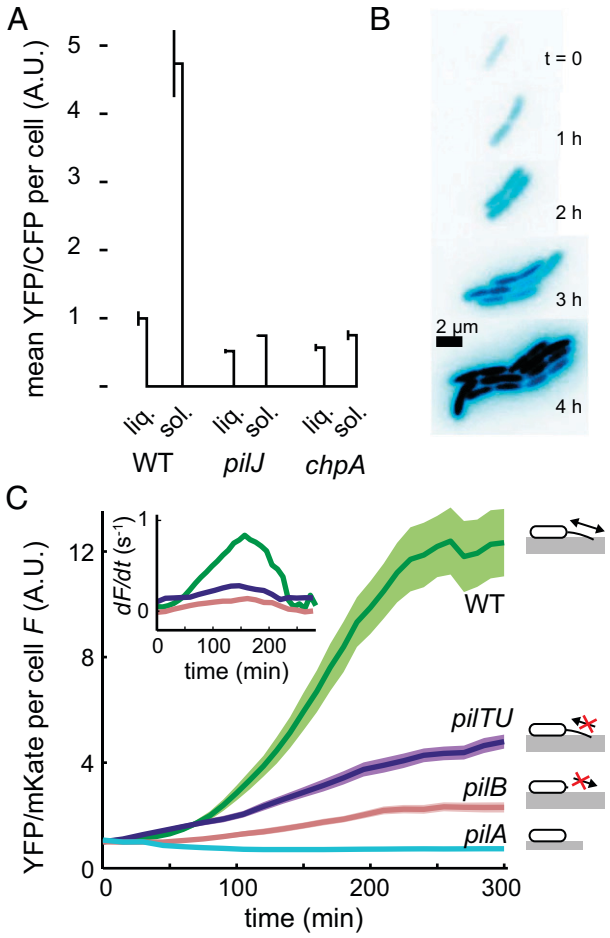

Fig. 2. The Chp chemosensory system and TFP extension/retraction mediate surface sensing. (*A*) The relative fluorescence intensity of the *PaQa* YFP reporter (normalized to *rpoD*-mKate) in WT versus Chp system mutants was compared during growth in liquid media or when associated with a 1% agarose hydrogel. Cells lacking PilJ or the histidine kinase ChpA failed to activate the *PaQa* reporter upon surface contact. Error bars represent 95% confidence intervals (n = 4). (*B*) The fluorescence intensity of *PaQa* YFP-expressing bacteria growing on an agarose pad was determined as they transition from a liquid culture. (*C*) The fluorescence intensity ratio *PaQa*-YFP:*rpoD*-mKate was measured for each cell in the colony grown on 1% agarose. Shown is the average ratio per cell as a function of time. WT colonies demonstrate a strong increase in *PaQa*-YFP, whereas mutants lacking TFP fail to activate *PaQa*-YFP. Solid lines represent the mean fluorescence over multiple colonies (n = 12), and shaded regions correspond to 95% confidence intervals. (*Inset*) The rate of increase in florescence (*dF/dt*) reveals a strong pulse in *PaQa* promoter activity that is absent in the *pilA* mutant and diminished in strains with defective TFP extension (*pilB*) and retraction (*pilTU*).

the Chp system do not twitch (17) and mutants in TFP components affect cAMP production (9). However, whether TFP could also activate Chp upon surface contact remained unknown. To test whether surface sensing requires TFP, we first measured the response of the *PaQa* reporter in cells lacking structural and functional TFP components. To quantitatively assess the contributions of the TFP motors to surface sensing, we used time-lapse microscopy and quantified the mean *PaQa* reporter fluorescence per cell of surface-associated colonies (Fig. 2*B*).

PilA is the major structural element of TFP; PilA subunit assembly powers TFP extension whereas disassembly drives retraction. The motor proteins for extension (PilB) and retraction (PilT and PilU) transduce chemical energy (ATP) into mechanical work by polymerizing and depolymerizing PilA (7). Disrupting the major pilin subunit, PilA, completely abolished sensitivity to contact with the hydrogel (Fig. 2*C*). This loss of sensitivity also demonstrates that flagella do not mediate this response, because *pilA* mutations do not affect the swimming motility system (19). Disrupting the TFP extension motor, PilB, or simultaneously disrupting the two TFP retraction motors, PilT and PilU, reduced the magnitude of the surface sensitivity compared with WT. Mutants lacking the PilTU retraction motors exhibited an increase in *PaQa* transcription compared with mutants lacking PilA or the PilB extension motor. Feedback regulation maintains PilA levels relatively constant in motor mutants such that these mutants' perturbed surface sensing can be attributed to altered motor activity. Thus, surface sensing requires PilA and is amplified by TFP extension and retraction. We note that in a simultaneous study Luo et al. showed that cAMP production increases in a PilA- and PilJ-dependent manner when *P. aeruginosa* are grown on an agar surface (10).

In a recent study we demonstrated that the TFP-associated protein PilY1 works with quorum sensing to stimulate virulence upon prolonged surface contact (5). Whereas PilB is required for the short timescale *PaQa* response investigated here, PilB was not required for the long timescale virulence response to surface association (5), suggesting that the Chp and PilY1 systems are distinct. To further distinguish the PilY1- and Chp-dependent surface responses, we characterized the effect of deleting *pilY1* on the surface sensitivity of the *PaQa* reporter. The *PaQa* reporter could still be induced by contact with agarose hydrogels in the *pilY1* mutant (Fig. S4). However, similar to the effect of PilY1 on its own expression (10), the magnitude of the *PaQa* induction was decreased in *pilY1* compared with WT. Therefore, PilY1 affects short timescale mechanosensation but is not absolutely required. This effect could be explained by the fact that *pilY1* mutants are partially defective in TFP assembly and retraction (20).

We further tested the possible dependence of *PaQa* induction upon surface contact on quorum sensing. On a solid substrate, a single cell grows into a dense colony (Fig. 2*B*), raising the possibility that high cell density activates quorum sensing-dependent genes via the LasR and RhlR regulons. To determine whether these regulators play a role in *PaQa* induction, we tested the effect of mutations in their corresponding genes on *PaQa* induction. Both *lasR* and *rhlR* mutants showed significant *PaQa* reporter induction upon surface growth (Fig. S5). The magnitude of the response was similar to that of WT cells, demonstrating that surface sensing is independent of quorum sensing despite the rapid increase in cell density. These results support the hypothesis that the short timescale initial surface association response is mediated by TFP and is independent from the long timescale response mediated by PilY1 and quorum sensing.

TFP Attachment to Surfaces Stimulates *PaQa* Expression. Given that TFP are necessary for mechanosensation and that their extension and retraction motors amplify this signal, we sought to determine how TFP are mechanically coupled to the surface. In particular, do TFP need to attach to a surface or is PilA required through some indirect mechanism? To determine whether more frequent TFP attachment stimulated surface sensing, we varied surface substrate density by exposing cells to engineered hydrogels with decreasing pore sizes (21). Here, smaller pore sizes lead to higher substrate density and thus a higher probability of TFP attachment upon extension. Time-lapse imaging of colonies enabled quantitative measurement of *PaQa* induction upon contact with the hydrogels of various pore size (Fig. 3). Fluorescence measurements showed that the increase in *PaQa* reporter fluorescence was slowest at the largest pore size (0.75% agarose), intermediate at the intermediate pore size (1%), and fastest at the smallest pore size (1.5%). We furthermore observed that the rates of change in fluorescence induction reach their maximum

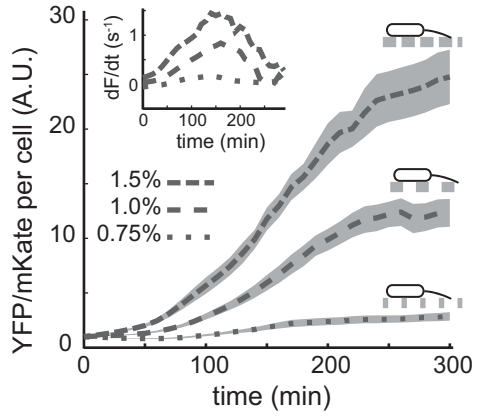

Fig. 3. The activity of *PaQa* upon surface contact depends on hydrogel mechanical properties. The relative fluorescence intensity of the *PaQa* YFP reporter (normalized to RpoD-mKate) per cell is averaged for each colony and reported as a function of time. Dashed lines represent the mean over multiple colonies ($n = 12$) and the shaded regions represent 95% confidence intervals. (*Inset*) The rate of change of fluorescence *F*. Higher agarose concentration yields hydrogels with smaller pore size, thus increasing interaction between TFP and the substrate.

values at ∼150 min on all three hydrogels, before returning to near-zero levels thereafter. This indicates that the differences of signal between the hydrogels are not due to a kinetic effect (i.e., where it takes longer to reach identical cAMP levels on larger pores), but rather on instantaneous sensing. The distinct steady-state value of *PaQa* induction on liquid and solid show that cAMP levels likely adapt to the distinct substrates (i.e., cAMP level is a memory of the attachment state of a cell).

When assembled, TFP undergo thermal fluctuations in the vicinity of the substrate (22). Attachment of a TFP tip depends upon encountering the agarose surface. We estimate that the timescale of TFP attachment scales with the diffusion time of its tip across the area of a pore. In our experiments, mean pore size varies between about 100 nm (1.5% agarose) and 300 nm (0.75%) (21). Therefore, in the range of agarose concentration tested here, the mean area of a pore decreases as the square of this difference, or roughly 10-fold between 0.75% and 1.5% gels. Balancing this area with the mean square displacement of a TFP tip (which scales approximately with time) shows that TFP attachment is roughly 10 times more frequent in the 1.5% gel compared with 0.75%, leading to stronger *PaQa* induction. We note that higher gel concentration results in harder gels, so that TFP possibly attach to and pull on stiffer gel fibers, which may also in part contribute to stronger signal (23). We thus conclude that *P. aeruginosa* directly senses TFP attachment as a means of sensing the extent of surface association, indicating that TFP coupled with Chp constitute a mechanosensitive machinery.

PilA Interacts with the Chemosensory Protein PilJ. How do TFP signal to the Chp system? We used the bacterial two-hybrid system (BACTH) to test the interaction between the two most likely candidates: PilJ, the MCP homolog, and PilA, the pilus subunit (Fig. S6*A*). PilA is associated with the inner membrane and has a very short cytoplasmic extension before being processed by a prepilin peptidase (7). PilJ has two transmembrane domains, and both its N and C termini are cytoplasmic (24). The BACTH system functions by using the interactions between two test proteins to reconstitute cAMP activity by bringing together the two subdomains (T18 and T25) of adenylate cyclase (AC) to reconstitute a functional enzyme. In the N terminus PilA fusion, the AC fragment will be localized in the cytoplasm (Fig. S6*B*). Both N- and C-terminal fusions of PilJ to either AC subunit should localize AC to the cytoplasm. Coexpression of the N-terminal PilA fusion with the N- or C-terminal T25 or T18 PilJ fusion, respectively, resulted in a large increase in AC activity (Fig. S6*A*). We verified that this signal was not a result of nonspecific interactions between the N-terminal fusion to PilA with another integral membrane protein by measuring the interaction with fusions to FimV (8), which were both negative (Fig. S6*A*). Also, fusions to PilJ lacking the transmembrane domains resulted in loss of signal (Fig. S6*A*). Together these results indicate that PilJ and PilA can interact. Because PilA does not have a cytoplasmic domain, PilA and PilJ likely interact through their periplasmic domains. The periplasmic domain of PilJ includes its chemosensory domain, indicating that the Chp system may respond to the PilA ligand as a signaling input.

Mechanotransduction Requires Both TFP Tension and Retraction. How could a PilA–PilJ interaction sense TFP attachment and induce surface mechanosensing? Attached TFP are under tension during retraction and this tension could modify TFP, for example by inducing a modification in PilA. PilJ could sense such tension-induced changes at the attachment point between the cell body and the TFP, where PilJ colocalizes (25). Alternatively, PilJ could sense TFP modification during retraction, as PilA subunits depolymerize into monomers and are incorporated back into the inner membrane. These two models differ in their dependence on retraction; if PilJ senses tension at the base of TFP, then in the absence of retraction, placing TFP under tension would restore signaling. In contrast, if stretched TFP activate PilJ as they retract, then tension in the absence of retraction would not further stimulate signaling. To differentiate these two models, we generated tension in TFP of a *pilTU* mutant, defective in TFP retraction (Fig. S7). We initially attached *pilTU* cells onto the glass surface of a microfluidic channel. Here, unretracted TFP allow for efficient attachment to glass. To generate tension in TFP of attached cells, we applied flow in the microchannel. Shear stress generates a force on the cell body that is parallel to the surface and is balanced by tension in TFP. We set the flow rate to generate roughly 10 pN of force, which is on the order of forces generated by TFP retraction (26).

We measured the response of the *PaQa* reporter in the *pilTU* background in the presence and absence of flow. Similarly to the case of growth on agarose pads, we measured an increase in the fluorescence intensity of the reporter upon attachment in both flow and no-flow conditions (Fig. S7). However, fluorescence intensities measured in flow and no-flow conditions had similar amplitudes, indicating that further TFP tension generated by flow does not increase the response. In the no-flow case, the response upon surface attachment may originate from mechanical perturbations, for example tension induced by Brownian motion of the cell body. Importantly, flow-induced TFP tension failed to restore *PaQa* promoter activity to the levels measured on agarose pads (Figs. 2*C* and 3). These results indicate that efficient PilJ signaling via TFP activity requires not only extension and attachment but also the internalization of stretched TFP, and refutes a mechanism where PilJ senses TFP tension without retraction. The residual induction observed in the absence of PilTU could be explained by interactions between PilJ and the small number of PilA subunits found at the base of the pilus. Such weak interactions would also explain the low level of *PaQa* induction observed in *pilB* mutants (Fig. 2*C*).

Discussion

We demonstrated that TFP not only function as a structure enabling surface motility, but also as a force transducer promoting the induction of virulence factors when cells encounter surfaces such as host tissues. Based on our results, we propose a mechanochemical model for surface sensing where TFP function both as mechanical actuators and sensors whose output is

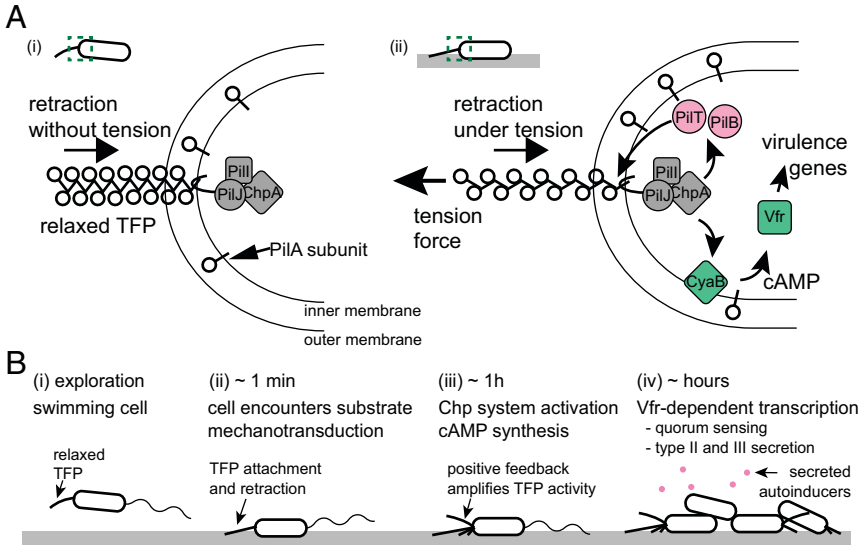

Fig. 4. Proposed molecular model for surface sensing mediated by type IV pilus. (*A*) The pilus extends and retracts by assembly and disassembly, respectively, of PilA monomers into a polymer. During growth in liquid, TFP do not attach, therefore TFP are not actuated upon retraction (*i*). Upon contact with a solid surface, attachment and retraction exerts tension on TFP leading to an uncharacterized modification (*ii*). This modulates the interaction between the periplasmic domain of the MCP chemosensory protein PilJ with PilA and transduces the signal to the cytoplasm: PilJ activates the CheA homolog ChpA complexed with the CheW homolog PilI, stimulating CyaB activity and cAMP production. cAMP thus activates the transcription factor Vfr, increasing transcription of a wide range of genes including virulence factors. Simultaneously, the Chp chemosensory system stimulates TFP extension/retraction via PilG and PilH response regulators, leading to a positive feedback of twitching and surface sensing upon contact. (*B*) A model for the initiation of Vfr-dependent transcription on abiotic surfaces. (*i*) Single planktonic cells possess background TFP activity as they swim in the bulk of the fluid. (*ii*) Upon substrate contact, extended TFP attach onto the surface. Retraction rapidly generates tensile load on the fiber. (*iii*) Mechanotransduction activates the Chp chemosensory system, leading to an increase in TFP extension and retraction frequency, and induction of cAMP production. (*iv*) With increased twitching motility and cAMP levels, cells simultaneously explore the substrate and activate Vfr-dependent genes, including type II and III secretion systems, potentially leading to acute infection of a host. Vfr also activates the quorum sensing system thereby stimulating the secretion of autoinducers.

chemically read out by the Chp system (Fig. 4*A*). As they swim in the bulk of a fluid, planktonic cells extend and retract TFP (27). When cells encounter a surface, their TFP adhere to the substrate. Retraction generates tension in the TFP between its anchor and the cell. This tension induces an unknown modification in TFP that the cell reads out upon depolymerization, through the interaction between PilA and PilJ. This explains why loss of PilA, the building block of the mechanical actuator, completely abolished surface sensitivity. The reduction of the number of retracted PilA in the *pilTU* mutants hindered response. In this mutant, extended TFP still maintain the ability to attach, and other factors such as flagellar propulsion may induce tension in nonretracted fibers. Thus, PilJ could interact with PilA at the stretched TFP base despite the lack of retraction, so *pilTU* mutants remain partially sensitive to surface attachment, but with reduced sensitivity. Inhibiting TFP extension in the *pilB* mutant nearly abolished mechanosensation, because a PilB mutant lacks surface TFP (17). PilA negatively regulates itself, so that *pilTU* and *pilB* mutants have similar levels of intracellular PilA, and likely similar periplasmic levels because *pilTU* maintains the ability to extend full-length TFP (17). The difference of signals measured in these two mutants is therefore an effect of TFP extension and retraction rather than an artifact generated by depletion of PilA.

Our findings suggest that *P. aeruginosa* measures mechanically induced modification in TFP to interpret its mechanical environment. In *Neisseria gonorrhea*, TFP exhibit conformational changes upon stretching (28) and stretching reduces TFP width. Interestingly, these changes expose hidden PilA epitopes, indicating that PilA itself may change conformation upon tension. In *P. aeruginosa*, measurements at the level of single cells also showed that stretching induces stable TFP conformational changes (29). Based on these measurements, we propose that such changes modulate the interaction between PilA and the sensor PilJ, possibly triggering phosphorylation of cytoplasmic response regulators by the Chp system. For example, changes in PilA conformation could alter its interaction with PilJ. Alternatively, a change in TFP width upon stretching could modify the spatial configuration between PilA and PilJ. In this scenario, PilJ might act as a ruler for TFP width.

P. aeruginosa seems to have evolved a sensory system that is specific to surface contact by coupling TFP to an MCP, two systems that are widespread in bacteria. TFP already mechanically couple the bacteria to the surface of substrate, and are repurposed here to sense attachment. PilJ activation then stimulates the Chp and CyaB signaling cascade, leading to an increase in intracellular levels of cAMP, thereby activating Vfr-dependent transcription. In turn, PilJ, an MCP-like chemosensory system that is typically used to measure changes in environmental compounds, transduces mechanically induced changes in TFP into a virulence response. The increased signaling observed on denser substrates suggests that the Chp system transduces input frequency (retraction frequency of TFP under load) into a continuous output (cAMP levels). Chemosensory systems are well suited to perform such discrete to continuous conversion, as exemplified by the modulation of swimming runs during ligand binding in chemotaxis. How the signal generated by a PilA–PilJ interaction is transduced into a transcriptional response remains to be elucidated. Our results suggest that the amplitude of the transcriptional response scales with the frequency of signaling events (i.e., how often PilJ senses TFP under tension). Simultaneously, the Chp system increases PilB and PilT activity (17), stimulating TFP extension and retraction, prompting a positive feedback loop that increases surface sensitivity and twitching motility.

The Chp system is unique because it repurposes a chemotaxis-like system to sense mechanically induced signals. In *P. aeruginosa*, the Wsp and PilY1-LasR systems also confer sensitivity to surfaces but are not activated by TFP activity and do not regulate cAMP-dependent genes (5, 30). Further biophysical characterization will

help decipher the role of mechanics in their activation. Additionally, other bacterial species have the ability to mechanically probe their environment. For example, a flagellum-dependent mechanism modulates transcription, surface motility, and biofilm formation in *Escherichia coli* (31). Inhibiting flagellum rotation potentially disrupts the flow of ions through the flagellar motor, thereby transducing a mechanical signal into a chemical signal. This system differs from our model in that it uses flagellar function as a mechanosensor and may be activated by swimming in fluids more viscous than water. In contrast, the system we describe here requires TFP to mechanically attach to a surface, a feature that is independent of fluid properties. The role of TFP as the mechanical component prevents nonspecific activation of the pathway by the chemical environment.

P. aeruginosa possess multiple surface sensing systems to coordinate various virulence pathways (Fig. 4*B* and Fig. S8). The mechanosensitive TFP system enables surface sensing on short timescales on abiotic surfaces. Swimming *P. aeruginosa* cells harbor low but significant numbers of TFP (27), enabling the ability to sense a surface upon contact with the substratum. Transient contact leads to activation of the Chp system, simultaneously stimulating cAMP production and TFP extension–retraction. Increased cAMP leads to Vfr-dependent transcription, activating the type II and III secretion systems (12). Vfr also regulates the LasR quorum sensing system and the minor pilin PilY1 (10, 32). Consequently, surface sensing by TFP increases sensitivity to quorum sensing, possibly activating other surface-specific virulence factors, for example via the synthesis of cyclic-di-GMP (5, 10). Thus, *P. aeruginosa* harnesses the TFP surface sensing system to rapidly initiate infections upon surface contact. On a longer timescale (multiple hours or days), cells grow into biofilms and induce other virulence factors (Fig. S8).

Activating virulence pathways in response to mechanical contact, in lieu of sensing specific chemical cues, could promote the ability of *P. aeruginosa* to infect a wide range of hosts. Inducing virulence upon association with abiotic surfaces may also help combat invasion by competing microbes, thereby promoting robust surface colonization. Most studies on bacterial development have focused on the role of chemical environments in shaping physiological adaptations. Mechanics are a ubiquitous feature of bacterial habitats; our results highlight the possibility that they actively sense mechanical forces to adapt to their environments.

Materials and Methods

To measure reporter activity, overnight cultures of PAO1 containing the *PaQa* reporter plasmid (*SI Materials and Methods* and Table S1) were diluted into fresh LB and grown at 37 °C to midexponential phase. One hundred microliters of culture was then spread and dried onto each hydrogels and incubated for 3 h at 37 °C. Cells were then harvested by washing the hydrogels and mechanically scraping their surface with 400 μL of PBS. We delivered a 1-μL drop of the harvested population onto an agarose pad then covered it with a no. 1.5 coverslip. Finally, we acquired phase contrast and fluorescence images of the cells (*SI Materials and Methods*). These images were then segmented using phase contrast and the cell contours were used as masks to measure the relative fluorescence of the *PaQa* transcriptional reporter to the reference (YFP/CFP). We measured this ratio for more than 100 cells per experiment and reported the average of four replicates with their 95% confidence intervals based on a *t* distribution.

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