ABSTRACT The mechanisms by which bacteria sense and respond to surface attachment have long been a mystery. Our understanding of the structure and dynamics of bacterial appendages, notably type IV pili (T4P), provided new insights into the potential ways that bacteria sense surfaces. T4P are ubiquitous, retractable hair-like adhesins that until recently, due to their nanoscale size, were difficult to image in the absence of fixation. This review focuses on recent microscopy innovations used to visualize T4P in live cells to reveal the dynamics of their retraction and extension. We discuss recently proposed mechanisms by which T4P facilitate bacterial surface sensing, including the role of surface-exposed PilY1, two-component signal transduction pathways, force-induced structural modifications of the major pilin, and altered dynamics of the T4P motor complex.

KEYWORDS Pseudomonas aeruginosa, biofilm formation, cyclic di-GMP, gene regulation, microscopy, surface sensing, type IV pili

Most bacteria live in communities associated with abiotic surfaces, including soil, rocks, and medical devices, or on biological surfaces, such as epithelia, teeth, and skin (1). The transition from a planktonic state to surface attachment mode leads to the differential expression of genes involved in bacterial physiology, morphology, and behavior, primarily those associated with motility and adhesion (2–5). Surface attachment is required for both host invasion and infection (6) but also for surface-associated behaviors, such as formation of biofilms, which are significantly more tolerant of antibiotics compared to their planktonic counterparts (7). Understanding how bacteria sense contact with surfaces is therefore important, as interfering with this process has the potential to impede infection. Both physicochemical and mechanical signals experienced by a bacterium in close proximity to a surface have been proposed (8).

Physicochemical cues that indicate changes in the environment, including osmolarity, pH, and nutrient availability, are detected by bacterial two-component systems (TCS) (8). A direct role for two-component systems in bacterial surface sensing has yet to be identified, but multiple two-component systems involved in surface-associated behaviors like biofilm formation have been characterized (9–11). Because bacteria can encounter a broad range of environments, the feasibility of a single or several two-component systems controlling surface sensing is limited, as unique responses may be required for distinct surfaces. Mechanical signals provide a more generic way for bacteria to sense and respond to a broad range of surfaces. This mechanism, termed mechanosensing, involves the detection of changes in the mechanical load on a bacterium upon surface attachment. Motor-driven bacterial appendages, including type IV pili (T4P) and flagella, have been implicated in bacterial surface sensing (12, 13). Mechanical load on the motor fluctuates when bacteria encounter changes in viscosity or tethering of appendages to surfaces, which can initiate intracellular signaling (14). Mechanosensing of physical cues such as surface stiffness or external forces has
previously been demonstrated for plant and mammalian cells (15, 16). However, uncovering the role of individual mechanosensors is difficult, as bacteria may simultaneously encounter several distinct mechanical cues at a surface (14).

Here, we focus on surface sensing facilitated by T4P, ubiquitous bacterial appendages involved in adhesion (17), twitching motility (18), bacteriophage infection (19, 20), DNA uptake (21, 22), and electron transfer (23). Mechanosensing via T4P offers several advantages, as T4P are free from attachment during planktonic growth and experience a significant increase in mechanical load on the motor during surface attachment. Surface detection at a T4P-defined distance ranging from 0.3 to 8 μm (24) could also initiate intracellular signaling prior to attachment of the cell body, resulting in rapid intracellular responses upon surface contact.

With their average diameter of ∼6 nm (25), live imaging of T4P in vivo is challenging, as their size is below the diffraction limit of standard light microscopy. Studies of T4P have thus relied on comparisons of piliated cells with nonpiliated or genetically modified systems or on static methods, such as indirect force spectroscopy (26–28) and electron microscopy (29). While these studies provide insight into T4P structure, the dynamics of T4P expression in vivo are lost. Recent innovative methods to directly visualize T4P have opened our eyes to the dynamics of these nanoscale appendages and will help to uncover the role of T4P in surface sensing.

**VISUALIZATION OF TYPE IV PILUS DYNAMICS**

**Cysteine labeling of T4P.** Direct observation of T4P dynamics in vivo was first enabled 20 years ago in *Pseudomonas aeruginosa* (30) through the use of succinimidyl ester dyes which label surface-exposed primary amines. That seminal study expanded upon previous optical tweezer (18) and surface-immobilized T4P (31) experiments to confirm the role of T4P in twitching motility—first proposed by Bradley (32)—which involves repeated cycles of T4P extension, surface attachment, and retraction, leading to movement along solid surfaces (33). However, this nonspecific labeling technique results in high background fluorescence and requires the use of specialized microscopy systems to resolve individual filaments (34).

To combat these limitations, innovative methods developed by Ellison et al. (12) use directed cysteine labeling of the major structural subunit of T4P to visualize their real-time dynamics (12, 21, 34). T4P are primarily composed of major pilin subunits that undergo repetitive cycles of polymerization and depolymerization from the inner membrane (IM) upon pilus extension and retraction, respectively (33, 35). Major pilins have a conserved lollipop-like structure with a highly conserved, extended N-terminal α-helix and variable, globular C-terminal domain (35). The new labeling method, first established to visualize the functional dynamics of tight adherence T4P in *Caulobacter crescentus* (12), involves introduction of site-directed cysteine substitutions at solvent exposed positions in the globular C-terminal domain of pilins (34). The modified T4P can then be fluorescently labeled with a thiol-reactive maleimide dye (12, 34). The contributions of single amino acid residues to pilin structure and function, as well as the relative surface accessibility of residues (36) must be considered, as mutations of nonconserved, solvent-exposed residues are more likely to produce functional labeled T4P (34). Although many type IV pilins contain a disulfide bond critical for their folding and polymerization, addition of a third Cys residue does not impact function if the site is carefully selected.

This labeling approach has been used successfully to visualize all three subclasses of T4P, namely, (i) the type IVc tight adherence (Tad) T4P in *C. crescentus* (12), (ii) the type IVa competence and mannose-sensitive hemagglutinin T4P in *Vibrio cholerae* (21, 34), (iii) the type IVb toxin coregulated T4P in *V. cholerae* (34), and, most recently, (iv) the type IVa T4P in *P. aeruginosa* (24). In contrast to previous labeling approaches, which require a specialized total internal reflection fluorescence (TIRF) microscopy setup to resolve individual T4P (30), cysteine-labeled T4P can be imaged using an epifluorescence microscope with a high numerical aperture oil objective and charge-coupled device (CCD) camera (34). Directed labeling of pilins results in minimal background fluorescence from the cell body, allowing for the visualization of shorter (<5 μm) T4P.
(24, 30), although labeled pilins accumulate in the cytoplasmic membrane following pilus retraction, leading to circumferential fluorescence. The amide labeling technique pioneered by Skerker and Berg (30) resolved P. aeruginosa T4P with an average length of 5 μm, whereas recent studies of cysteine-labeled T4P in C. crescentus (12) and P. aeruginosa (24) revealed average pilus lengths of 1.08 μm and 0.8 μm, respectively. The latter labeling technique also provided new insight into the proportion of piliated cells in a population, as well as the number of T4P produced per cell over time. While electron microscopy images of P. aeruginosa suggested that only 25% of surface-grown bacteria are piliated (37, 38), live imaging of cysteine-labeled, surface-grown P. aeruginosa revealed that >80% of the bacterial population is piliated, with an average of approximately eight T4P produced per minute (24). Surprisingly, the majority of these T4P were less than 1 μm, a length not previously detected by dynamic or static microscopy methods (30, 37, 38). C. crescentus produced on average two T4P per minute (12), suggesting that pilus dynamics are variable among surface-associated bacterial species.

Pilus extension and retraction have traditionally been studied using nonfunctional or genetically modified T4P machinery, primarily knockouts of the extension and retraction ATPases. However, mutations in the retraction ATPase PilT result in hyper-piliated phenotypes and altered retraction and extension dynamics, as reported for P. aeruginosa and Neisseria species (39, 40). Most species which express toxin-coregulated pili (TCP), including V. cholerae (41), and those that express type IVc pili, including C. crescentus (33), lack a retraction ATPase orthologue, limiting the use of deletions to study T4P dynamics (34). Blocking TCP retraction in V. cholerae through point mutations in minor pilins, low-abundance proteins essential for T4P assembly (42), has been reported (41). However, disruption of pilus dynamics by deletion of the retraction ATPase PilT has global effects on transcription in Neisseria gonorrhoeae (40). To prevent unintentional downstream effects caused by mutations, Ellison et al. developed two methods to sterically disrupt pilus retraction in real-time in wild-type cells (12, 21). In this approach, cysteine-labeled T4P are treated with maleimide-conjugated molecules that interfere with pilus dynamics (34). The first method, originally used to disrupt type IVc pilus function in C. crescentus, involves the labeling of pili with a bulky polyethylene glycol-maleimide conjugate that is too large to pass through the secretin, stalling pilus retraction (34). The second method uses a two-step incubation with a biotin-maleimide conjugate followed by a streptavidin derivative and has been used to interfere with function of type IVa pili in V. cholerae (12). An additional incubation step to obstruct type IVa pilus retraction may be necessary due to the larger pilin size of type IVa pilins versus type IVc pilins, and labeling other T4P subclasses may need optimization (34). It should be noted that these labeling methods could have additional effects on cells, so it is important to control such experiments using a wild-type control that lacks the cysteine knock-in.

**Interference-based microscopy.** Among the drawbacks of labeling T4P with reactive cysteines is the potential disruption of pilin structure and signaling due to mutations or modifications, as well as the photobleaching of maleimide dyes during long-term imaging (43). A less invasive imaging technique which was recently used to visualize type IVa T4P in P. aeruginosa is interferometric scattering microscopy (iSCAT) (43). iSCAT is a light scattering-based imaging technique that enables the visualization of nanoscale particles and proteins without the use of fluorescent molecules or dyes (Fig. 1). iSCAT detects nonfluorescent scattered light caused by the interference between two coherent incident waves as they encounter an object of a different refractive index (44). Scattered light from nanoscale structures is amplified by an additional known reference field to resolve single entities, including viral particles (45) and single proteins (46). Compared to other superresolution microscopy techniques, iSCAT is relatively low cost and is easy to implement on existing microscopy systems (44). iSCAT overcomes the optical limitation of photobleaching, permitting long-term imaging of biological samples (44) and fast live-imaging speeds of up to 40 kHz while maintaining nanometer precision (47). In contrast, previous in vivo studies of T4P permitted time-lapse images on the order of seconds (12, 21, 24, 30). The rapid imaging speed of iSCAT
allows for near-real-time visualization and quantification of successive pilus extension and retraction events (43).

The reliance of iSCAT on the detection of scattered light resolves both the position and orientation of nanoscale bacterial appendages in three dimensions (43, 48). Unlike epifluorescence or TIRF microscopy, iSCAT distinguishes between T4P which lie flat along a surface and those attached at an angle, based on periodicity in the contrast of the appendage (43). This added dimension can distinguish between pilus movements associated with standing cells versus horizontal cells, as well as detached T4P (43, 49). The position and orientation of T4P are determined from the pattern of light and dark contrast along the length of the pilus, representing regions of constructive and destructive interference, respectively (43, 44). Visualization of *P. aeruginosa* T4P using iSCAT revealed the following three distinct T4P patterns: (i) T4P that lie flat on a surface with constant contrast, (ii) T4P attached to a surface at an angle with alternating dark and light contrast, and (iii) detached filaments with faint contrast (43). One limitation of iSCAT is that, similarly to the amide labeling technique pioneered by Skerker and Berg (30), the strong signal from the cell body generates a halo that obscures T4P shorter than ~3 μm (24, 43). Thus, a combinatorial approach of real-time epifluorescence microscopy of cysteine-labeled T4P and iSCAT imaging of nonlabeled T4P allows for direct visualization of the entire T4P population, as well as the position and orientation of longer (>3-μm), unmodified T4P (24, 43).

**POTENTIAL MECHANISMS OF PILUS-MEDIATED SURFACE SENSING**

Innovations in the optical detection of T4P reveal their patterns of expression, length, position, and orientation in vivo (12, 21, 24, 43). However, the exact manner in which T4P relay information in surface sensing is still unclear. Among the puzzles that remain to be solved are the differences between the properties of— and responses to— pili that are extended and retracted without surface attachment (and thus are not under tension) versus filaments that extend and bind to a surface. Here, we present recent studies aimed at trying to uncover mechanisms underlying bacterial surface sensing, which highlight potential roles of the nonpilin protein PilY1, two-component signal transduction pathways, the Pil-Chp chemosensory system, the T4P motor complex, and force-induced conformational changes in the major pilin (Fig. 2).

**PiliY1 and related adhesins.** Pathogenic bacteria rely on surface attachment to induce virulence, as host colonization is contact dependent (50–52). The large surface-exposed component PilY1 and its homologues are conserved across a broad spectrum of pathogenic bacteria, including *Neisseria* (where there are two paralogues, PilC1 and PilC2) (53, 54), *Xanthomonas* (55), *Xylella* (56), *Pseudomonas* (50), *Legionella* (57), *Burkholderia*, and *Acinetobacter* species (50), and it has been identified as a putative mechanosensor in *P. aeruginosa* (50). PilY1 is a putative pilus tip adhesin that contains
a region near the N terminus with limited sequence similarity to the mechanosensitive von Willebrand factor A (VWFa) domain in eukaryotes (50, 58–61). This domain was first identified in prokaryotes in the Streptococcus agalactiae PilY1 homologue and is essential for epithelial cell adhesion (62). In P. aeruginosa, deletion of the VWFa domain "activates" PilY1 and increases the virulence of planktonic cells against amoebae (50).

The shear force from surface attachment is hypothesized to partly unfold this region of PilY1. This signal is then propagated along the length of the pilus in an unknown manner to a periplasmic or membrane sensor protein, leading to downstream upregulation of virulence factor expression (50). Signaling could occur via activation of the diguanylate cyclase, SadC, to increase c-di-GMP levels (38, 63–65) or via the minor pilin-regulated TCS FimS-AlgR (66, 67). The consequences of specific mutations must be considered when evaluating phenotypes, as pilY1 deletion strains are nonpiliated, making it difficult to dissociate phenotypes associated with loss of T4P assembly and function from those related specifically to loss of PilY1 (50). Complementation of pilY1 mutants using multicopy plasmids has additional consequences, as PilY1 and its partners PilVWX negatively control their own expression via the FimS-AlgR TCS. Overexpression of these components in trans represses expression of genes in the broader AlgR regulon, while deletion of minor pilins or PilY1 leads to AlgR activation (66).

**Two-component systems.** Multiple TCSs, primarily those involved in virulence and biofilm-associated phenotypes, are upregulated upon surface attachment (2–5). TCSs
linking surface sensing to bacterial appendages have been identified, including DegS-DegU in *Bacillus subtilis*, which is activated by loss of flagellar rotation (68, 69), and FimS-AlgR in *P. aeruginosa*, which contributes to upregulation of T4P expression upon surface contact (64). However, the potential role of major pilin expression in surface sensing has yet to be explored. The inventory of pilins in the IM fluctuates as pilins are extracted and incorporated into a pilus fiber then subsequently returned to the IM upon pilus retraction (70, 71). Expression of the major pilin is tightly controlled by the PilIS-PilR TCS in several T4P-expressing bacteria (70, 72–77), although it is best characterized in *P. aeruginosa*.

The *P. aeruginosa* PilSR TCS regulates the inventory of the major pilin, PilA, through a phosphorelay between the IM histidine kinase, PilS, and the cytoplasmic response regulator, PilR (70, 78, 79). PilA interacts directly with PilS, promoting its phosphatase activity on PilR, leading to downregulation of *pilA* transcription. Conversely, decreased interactions with PilA promotes the kinase state of PilS, activating PilR and upregulating the transcription of *pilA* and other PilR-regulated genes (70, 80). Transcriptomic analysis of *pilA* and *pilR* mutants (80) led to the identification of 10 genes that may be responsive to pilin inventory in the IM, including two structural components of the *P. aeruginosa* H2 type VI secretion system (T6SS), which are involved in interbacterial competition and host interactions (80–82). T4P-mediated surface attachment likely leads to a transient drop in PilA inventory, as pilin subunits are trapped outside the cell in a tethered pilus (33). This transient reduction in PilA-PilS IM interactions when a pilus fails to retract could activate PilR and its regulon, including *pilA* and components of the T6SS (80, 82). Signaling through the PilIS-PilR TCS in response to fluctuating IM pilin stores is a potential way to detect surface attachment, upregulating both T4P and other virulence factors (80). However, recent analysis of pilus extension and retraction dynamics (24) found that in *P. aeruginosa*, a typical pilus dwells on a surface for less than 1 s. This time frame may not allow for both sensing and response to a transient loss of PilA in the IM. For example, in *Porphyromonas gingivalis*, the T9SS-associated TCS PorY-PorX autophosphorylates within 1 min of exposure to environmental stress in vitro (83). There is an additional 6- to 8-min interval between phosphorylation and promoter response, as seen in the *Escherichia coli* TCS PhoR-PhoB (84). A delay of up to 9 min in the pilin promoter response does not align with a typical pilus dwell time of less than 1 s, suggesting that other signaling pathways facilitate bacterial surface sensing.

**Pil-Chp chemosensory system.** The Pil-Chp chemotaxis-like sensory system responds to mechanical cues associated with T4P extension and retraction (85). This complex signal transduction pathway controls T4P-mediated twitching motility and regulates cAMP production through activation of the adenylate cyclase, CyaB (64, 86–88). Increases in intracellular cAMP activate the transcription regulator Vfr (virulence factor regulator), which controls the expression of more than 100 genes mostly involved with pathogenesis, including those related to T4P biogenesis (88). Nonfunctional mutations or deletions in the Chp system or the Vfr signaling cascade reduce twitching motility and T4P biogenesis, although this is independent of pilin expression (85, 89, 90). PilJ, a methyl-accepting chemotaxis protein (MCP)-like chemosensory receptor, undergoes a conformational change in response to poorly characterized stimuli that promotes autophosphorylation of the kinase ChpA (91). ChpA, in complex with PilI, relays this phosphate to PilG, which stimulates cAMP production, or PilH, which reduces cAMP production (91).

Persat et al. (85) found that PilJ interacts directly with PilA, likely through their periplasmic domains. Interestingly, interactions between PilJ and PilA require contact with a surface and subsequent retraction, suggesting that pilins undergo an unknown conformational change when under tension that permits PilJ binding (70, 85). The differential binding of PilJ to PilA under tension versus that to relaxed PilA may facilitate T4P-mediated surface sensing on a more reasonable time scale (~1 s) compared to the PilIS TCS (85). When bound to surface-attached pilins, PilJ promotes CyaB activity via ChpA, leading to subsequent activation of Vfr and its regulon (Fig. 2) (85). Vfr-mediated feedback positive regulation of T4P expression promotes further pilus biogenesis upon
surface contact, promoting long-term surface adhesion (90, 92). Recent structural reconstructions of major pilins have identified conserved domains in the N-terminal \(\alpha\)-helix, which may contribute to the differential binding of PilU (92, 93).

**Force-induced pilin modifications.** To accommodate retraction forces upwards of \(~100\) pN (91), *N. gonorrhoeae* T4P can be reversibly stretched to roughly three times their length (94). Stretching of T4P in response to mechanical stress has also been observed in other families of filaments, including CS1 T4P in enterotoxigenic *Escherichia coli* (95). Structural studies of the major pilin, PilE, in *N. meningitidis* revealed conserved features in the N-terminal \(\alpha\)-helix which allow for transition to this stretched state (92, 94–97). The 53-amino-acid N-terminal \(\alpha\)-helix (\(\alpha_1\)) of PilE is divided into two sections, \(\alpha_1\)-N and \(\alpha_1\)-C (92, 96–99). The variable \(\alpha_1\)-C domain extends to the globular C-terminal \(\beta\)-sheet, while the highly conserved \(\alpha_1\)-N helical domain anchors pilins in the inner membrane (94, 95, 98, 99) and forms extensive interactions with other \(\alpha_1\)-N domains in the assembled pilus (96, 97, 100, 101). Major pilins have a characteristic S-shaped N-terminal \(\alpha\)-helix caused by two helix-breaking residues in \(\alpha_1\)-N, Pro22 and Gly/Pro42, which are widely conserved in type IVa T4P-expressing bacteria (99, 102). An additional glycine residue at position 14 is conserved (92).

X-ray crystallography and cryo-electron microscopy (cryoEM) reconstructions of *N. meningitidis* PilE filaments showed that \(\alpha_1\)N becomes unstructured between Gly14 and Pro22 upon assembly (92). Gly14 and Pro22 may destabilize the secondary structure of the pilin, allowing the intervening segment to melt (92). In contrast, pilins anchored in the inner membrane have an entirely helical \(\alpha_1\)-N (90). This phenomenon was seen in cryoEM reconstructions of both *P. aeruginosa* and *N. gonorrhoeae* pili (93), as well as in PuLG, the major pilin of type II secretion system pseudopili (103). The restructuring of this flexible segment is proposed to allow for efficient packing of pilin subunits and contribute to filament flexibility (92, 93). Mechanical stress on the pilus forces this melted segment into an extended state, resulting in a spring-like extension of the pilus (92, 102, 103). Force-induced stretching of PilE exposes key residues at the end of \(\alpha_1\)-C, which is involved in *N. meningitidis* attachment to the endothelial cell receptor, \(\beta_2\)AR (92, 104, 105). *N. meningitidis* pili activate \(\beta_2\)AR upon attachment, leading to loosening and opening of the blood brain barrier (104). Similar force-induced conformational changes have been observed in the mannose-binding adhesin FimH in *E. coli* type I pili to strengthen catch-bonds and enhance surface attachment (106). The presence of helix-breaking residues in \(\alpha_1\)N is conserved across type IVa pilins and type IVb pilins (92, 107), suggesting that force-induced changes in pilin conformation may have an important function in pilin signaling, potentially through interactions with PilU. It is plausible that PilU binds to residues in the “melted” portion of PilA, flanked by Pro22 and Gly42, which are exposed upon force-induced stretching of PilA.

**The type IV pilus motor complex.** A key unanswered question in the T4P field is whether the switch between pilus extension and retraction is stochastic or induced in the presence of a surface. Characterizing a conserved role for the T4P motor complex in surface sensing has proven difficult due to the variable number of motor ATPases in T4P-expressing bacteria. Type IVc Tad Tad pili of *C. crescentus* (41) and TCP of *V. cholerae* (106, 107) are retractile, despite a lack of a dedicated retraction ATPase. Similarly, in mutants of *N. gonorrhoeae* and *M. xanthus* lacking the retraction ATPase, T4aP retain partial function (108, 109). *P. aeruginosa* expresses three motor ATPases. PilIB is the pilin polymerase, PilT is the pilin depolymerase, and PilU is a PilT parologue whose function remains unclear (110–112). The tools developed to visualize T4P dynamics in real time (12) provided evidence for both coordinated and stochastic binding of the extension and retraction motors. In *C. crescentus*, loss of T4P retraction through surface attachment or perturbation with a bulky polyethylene glycol (PEG)-conjugated maleimide stimulated the surface contact-dependent production of holdfast polysaccharide (12). The production of holdfast in response to both surfaces and labeling with PEG-maleimide compounds suggests that disruption of T4P motor dynamics, rather than a mechanosensory signal on the surface itself, stimulates surface sensing (49). Upon
surface contact, Tad pili in *C. crescentus* act synergistically with flagella by positioning the cell body into an upright position after attachment (111) to promote flagellum-mediated surface sensing and stimulate holdfast production (113).

In *P. aeruginosa*, visualization of T4P dynamics with cysteine labeling and iSCAT provided conflicting results on the role of surface contact in stimulation of pilus retraction. Koch et al. (24) found that surface contact had no effect on T4P dynamics, with retraction events occurring in 95% of liquid-trapped and 93% of surface-attached cells. The dwell time between pilus extension and retraction, as well as the distribution of T4P lengths, were also indistinguishable between planktonic and surface-associated bacteria (24, 85). Although T4P dynamics were not altered by surface contact, changes independent of the motor complex—such as specific interactions of surface-attached major pilins with the MCP PilJ of the Chp system—were proposed to facilitate the detection of surfaces.

Stochastic activity of the T4P extension and retraction motors does not correlate with T4P dynamics recorded using iSCAT. Tala et al. (29, 43) found that *P. aeruginosa* precisely coordinates T4P motor activity, whereby surface contact via the tip of the pilus generates tension along the length of the filament prior to detachment. In contrast to Koch et al. (24), most extended T4P did not retract unless the tip of the pilus encountered and attached to a surface. This suggested that tension initiated from the bound tip of the pilus transduces an unknown signal that triggers retraction via PilT (43, 112, 113). The PilT paralogue PilU was required for T4P retraction along higher-friction surfaces, consistent with reports that it functions as a *bona fide* PilT-dependent retraction ATPase (43, 114, 115). This further suggests that the T4P motor complex can sense surface attachment, as it recruits an additional retraction motor to facilitate twitching under higher loads (43).

**CONCLUSIONS**

Our understanding of bacterial surface sensing facilitated by T4P has improved tremendously through recent innovations in the optical detection of these nanoscale filaments. These were made possible by applying a cysteine-labeling technique for flagella to T4P, as well as use of label-free interference-based microscopy methods to visualize T4P dynamics. Other major achievements are the structural characterization of T4P and its components using static but high-resolution imaging methods, including cryoEM, which revealed conserved features in T4P filaments. These structural motifs are thought to undergo conformational changes in response to shear force. A topic requiring further investigation is the identification of if—and how—these force-induced conformational signals propagate a mechanical or physicochemical signal via T4P filaments to indicate bacterial surface attachment. The obstruction of such a signal could have therapeutic implications to prevent host attachment and colonization of bacterial pathogens.

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