Published in final edited form as:

Curr Opin Microbiol. 2015 December ; 28: 78–82. doi:10.1016/j.mib.2015.07.017.

Cross-regulation of *Pseudomonas* motility systems: the intimate relationship between flagella, pili and virulence

Barbara I. Kazmierczak, MD, PhD^{1,2}, Maren Schniederberend, PhD², and Ruchi Jain, PhD²

¹Department of Microbial Pathogenesis, Yale University School of Medicine, 333 Cedar St., New Haven, CT 06520-8022, USA

²Department of Medicine (Infectious Diseases), Yale University School of Medicine, 333 Cedar St., New Haven, CT 06520-8022, USA

Abstract

Pseudomonas aeruginosa navigates using two distinct forms of motility, swimming and twitching. A polar flagellum and Type 4 pili power these movements, respectively, allowing *P. aeruginosa* to attach to and colonize surfaces. Single cell imaging and particle tracking algorithms have revealed a wide range of bacterial surface behaviors which are regulated by second messengers cyclic-di-GMP and cAMP; the production of these signals is, in turn, responsive to the engagement of motility organelles with a surface. Innate immune defense systems, long known to recognize structural components of flagella, appear to respond to motility itself. The association of motility with both upregulation of virulence and induction of host defense mechanisms underlies the complex contributions of flagella and pili to *P. aeruginosa* pathogenesis.

Introduction

Pseudomonas aeruginosa is a Gram-negative opportunistic pathogen associated with freshwater and soil reservoirs. In humans, it causes a broad range of infections associated with epithelial barrier disruption (corneal infections and keratitis, burn superinfections, ventilator-associated pneumonia), immunocompromise (neutropenic septicemia), and the genetic disease Cystic Fibrosis. Motility and attachment facilitate *P. aeruginosa* exploitation of and adaptation to these varied environments, and have been extensively studied. In this review, we will focus on new findings regarding the regulation of flagellar and Type 4 pilus assembly and motility. Changes in motility are often associated with other phenotypic changes that alter bacterial behavior and virulence toward hosts, and these will be highlighted throughout.

The transition between planktonic and sessile growth of *P. aeruginosa* is often depicted as a cycle of regulated events that allow bacterial populations to sense and adapt to surface-associated growth. Swimming bacteria arrive at a surface, undergo a transition from

reversible to irreversible attachment that is accompanied by changes in flagellar function [1], production of Type 4 pili (T4P) [2], intracellular accumulation of the second messenger cyclic-di-GMP (c-di-GMP) [3], and production of adhesins and exopolysaccharides that contribute to the formation of an organized biofilm community [4]. The recent application of particle-tracking algorithms to the analysis of *P. aeruginosa* motility has complicated our view of this process by showing the wide range of behaviors that occur at the single-cell level as bacteria come to a surface.

Coming to grips: initial encounters of *P. aeruginosa* with a surface

P. aeruginosa motility in low viscosity liquids is powered by a single polar flagellum. Only ca. 10% of such liquid grown bacteria assemble polar T4P [2], and so it is not surprising that initial binding of *P. aeruginosa* to surfaces is observed to occur via the spontaneous attachment of the polar flagellum [5,6]. Attached, vertically oriented bacteria spin (a flagellar-mediated movement), occasionally cartwheel (a T4P-mediated movement), and often detach. Bacteria that assume a horizontal orientation vis a vis the surface are more likely to remain attached, especially if they lack T4P (*pilA*). This somewhat paradoxical observation may indicate a role for T4P in pulling horizontal cells to a vertical orientation, where the probability of detachment is greater. Conrad *et al.* also followed the fates of post-division cells and found that >99% of siblings exhibit discordant motility behaviors, with one sibling remaining horizontally attached, while the second detaches, crawls or “walks” away from the division site. *pilA* bacteria do not move after division, indicating that postdivision motility requires T4P, while *fliM* (aflagellate) bacteria are more likely to move away after division (60%) compared to wild-type cells (40%) [5].

The tremendous heterogeneity of motility behaviors revealed by Conrad *et al.* highlights the additional information provided by single cell vs. bulk analyses of motility, but also raises questions of how such variation is achieved. A recently developed FRET-based biosensor for visualizing c-di-GMP concentration within individual *P. aeruginosa* cells may provide part of the answer. Using this tool, Miller and colleagues demonstrated asymmetry of [c-di-GMP] in daughters following cell division [7]. These differences in [c-di-GMP] result from asymmetric localization of a c-di-GMP phosphodiesterase (PDE) Pch (PA5017) to the flagellar pole, via direct or indirect interaction with the chemotaxis protein CheA. The authors postulate that c-di-GMP heterogeneity may generate many different motility phenotypes within a population, and demonstrate that ectopic expression of a PDE, which further lowers intracellular c-di-GMP, results in a greater mean swimming velocity and a greater number of flagellar reversals than exhibited by the population of wild-type *P. aeruginosa*. Conversely, the few *pch* bacteria that were observed to swim did so with a slower mean velocity and exhibited fewer reversals [7]. In many bacterial species, sensors that regulate flagellar rotation in response to c-di-GMP have been described. These include the PilZ-domain protein YcgR of *Escherichia coli*/Salmonella [8,9], which is postulated to disrupt motor-stator interactions and slow the flagellar rotor upon binding c-di-GMP. The c-di-GMP responsive regulator of flagellar rotation in *P. aeruginosa* appears not to be the YcgR homolog PA3353, however, as deletion of this gene had no effect on *P. aeruginosa*'s response to changing of c-di-GMP levels in these experiments [7].

Making a commitment to stay put

It is not yet clear how surface binding is “sensed” by *P. aeruginosa*, and it is likely that there are many independent mechanisms that trigger surface-associated behaviors in this organism. For some bacteria, it appears that the inhibition of flagellar rotation leads to a developmental switch that favors bacterial adhesion, such as assembly of a holdfast by *Caulobacter crescentus* [10] or production of exopolymers by *Bacillus subtilis* [11]. In each of these instances, flagellar surface binding is likely to impose a severe load on the flagellum that stops rotation: in the case of *B. subtilis*, contact by several of the peritrichous flagella may suffice, while surface contact by the polar pili of *C. crescentus* stops rotation of this organism's single polar flagellum. Experimental conditions that artificially stop flagellar rotation, such as the addition of anti-flagellin antiserum or polymers such as dextran or Ficoll, can trigger these developmental “switches” to attachment, suggesting that these bacteria respond to flagellar stalling. No studies published to date suggest that *P. aeruginosa* responds to flagellar stalling, but a few older papers have linked changes in the frequency of flagellar reversals with defects in biofilm formation [12]. The mutations that decrease biofilm formation increase swarming, a form of motility powered by flagella and modulated by T4P in *P. aeruginosa* [13], and map to *sadB* and *pilJ*. The mechanism by which SadB and PilJ could modulate flagellar reversal frequency is still not known, but it is interesting to note that PilJ is the methyl-accepting chemotaxis protein (MCP)-like component of a chemotaxis cluster linked to T4P regulation, Pil/Chp (reviewed [14]).

P. aeruginosa upregulates expression of T4P upon surface binding [2], and uses these nanomachines for twitching motility, a form of surface locomotion in which repeated cycles of extension, attachment and retraction of pili allow bacteria to crawl along a surface. Rapid reorientation of twitching bacteria can follow the sudden release of one T4P while other pili remain under tension, and the frequency of this “slingshot” like movement is affected by surface properties such as softness [15,16]. T4P genes are positively regulated by the cAMP-binding transcriptional regulator Vfr, while the function of T4P is under the control of the Pil/Chp chemotaxis cluster (recently reviewed by [17]). Mutation of Pil/Chp proteins disrupts T4P mediated motility and also alters intracellular cAMP levels, suggesting that T4P function might be linked to the synthesis of this second messenger. This relationship has been examined in recent papers, which track the behavior of surface-attached bacteria on different timescales. A Vfr-driven transcriptional reporter, *P_{PaQa}::yfp*, was used to demonstrate induction of cAMP-dependent transcription shortly (within 30–45 min) after *P. aeruginosa* bound to surfaces [18]. This transcriptional response was absent in a mutant that does not produce the T4P subunit pilin (*pilA*), attenuated in the absence of the retraction ATPases (*pilTU*) and markedly diminished in the absence of the assembly ATPase (*pilB*). External tension on T4P, generated by flow applied to attached *pilTU* bacteria, had no measurable effect on *P_{PaQa}* reporter activity, consistent with a model in which pilus retraction is a required aspect of mechanosensing. Based on interaction between PilA and PilJ in a bacterial two-hybrid system, Gitai and colleagues postulate that PilJ—the MCP-like component of the Pil/Chp system—responds to PilA as the T4P is retracted. The authors speculate that the tension placed upon an attached pilus as it retracts may physically modify or stretch T4P and potentially expose novel epitopes on PilA [19], thereby allowing PilJ to

respond specifically to attachment. A concurrent study, using a cAMP-Vfr dependent P_1 -*lacZ* reporter, likewise observed that bacteria increased cAMP levels when grown on solid agar in a T4P dependent manner [20]. PilA and PilJ were absolutely required for this upregulation, which was measured 5h after bacterial surface inoculation.

Tracking of bacteria at the surface of a flow cell over longer time periods (20–40h) revealed a role for PilY1, a surface adhesin upregulated by Pil/Chp and cAMP-Vfr, in irreversible surface attachment, experimentally defined as a horizontal orientation of bacteria on a flow cell surface [20]. Encoded within a polycistronic operon along with minor pilins, PilY1 is secreted to the cell surface via the T4P machinery (and possibly a T4P-independent pathway) where it activates the SadC diguanylate cyclase and increases c-di-GMP production [21]. The mechanism by which PilY1 activates SadC is not known. On the time scale of these experiments, *P. aeruginosa* colonizes surfaces via T4P mediated twitching motility, laying down trails of the exopolysaccharide Psl that subsequent bacteria tend to follow [22]. These trails guide bacterial exploration of the surface and lead to a “hierarchical”, rather than uniform, distribution of bacteria into aggregates and microcolonies. Interestingly, Psl also serves as a signal for c-di-GMP production by activating the diguanylate cyclases SadC and SiaD [4]. Whether PilY1 plays a role in Psl-mediated signaling to SadC remains to be addressed, but this c-di-GMP signal further upregulates production of biofilm-promoting protein and exopolysaccharide adhesins [4].

Virulence: the nasty side of surface associated *Pseudomonas*

Surface attachment is often thought of as a first step on the path to biofilm formation, but many recent papers have focused on ways in which the surface attachment of motile bacteria affects *P. aeruginosa* virulence. An ability to use T4P for twitching motility allows *P. aeruginosa* to move “upstream” against flow in microfluidic devices [23]. In branched microfluidic systems, twitching motility allows *P. aeruginosa* cells to move perpendicular to the direction of flow and gain access to side branches. Competing bacteria like *Escherichia coli* and *Proteus mirabilis*, which can swim upstream but lack pilus-mediated surface motility, can be seeded into a branched system along with *P. aeruginosa* and tracked by time-lapse microscopy. These faster growing bacteria outcompete *P. aeruginosa* in the seeded branch, but cannot access side branches, allowing *P. aeruginosa* to establish a protected niche within the system (Figure 1). T4P motility allows bacteria to move “upstream” through the vascular system of an inoculated plant leaf, suggesting that the surface-mediated motility may be relevant to dispersal through a host tissue. Whether the ability to twitch influences the spatial distribution of *P. aeruginosa* within a polymicrobially infected tissue, such as the chronically colonized Cystic Fibrosis lung, is an interesting point raised by this work.

Upregulation of cAMP and c-di-GMP production by surface-attached bacteria might be expected to have far-reaching consequences on the behavior of *P. aeruginosa*, given the established roles of these second messengers in regulating multiple virulence traits. In a direct test this hypothesis, *P. aeruginosa* cells grown in a shaking Petri dish were separated into planktonic and surface-attached populations and placed into contact with the amoeba *Dictyostelium discoideum* [24]. Calcein-AM fluorescence was tracked as a marker of

amoeba membrane compromise (and therefore killing) and revealed that the attached *P. aeruginosa*, but not planktonic cells, were able to rapidly (within an hour) kill amoeba. The mediators of killing were not identified by the authors, who showed that mutation of any of the systems associated with *P. aeruginosa* virulence did not abolish amoeba death (including Type 3 and Type 6 secretion, flagellar and Type 4 pilus biogenesis, exopolysaccharide production, surface-activated diguanylate cyclases, and quorum sensing systems), with the sole exception of Las quorum-sensing mutants. This finding, perhaps reflecting the activation of multiple, redundant virulence pathways in surface associated bacteria, suggests that the sensors required for this upregulation might be better targets for disrupting *P. aeruginosa* virulence than any individual virulence factor. O'Toole and colleagues demonstrate that surface virulence does not develop in bacterial populations lacking *pilY1*, suggesting that it and Las quorum sensing provide two signals—surface attachment and cell density—that promote expression of virulence in a host-nonspecific manner [24].

Host immune responses to *P. aeruginosa* motility

It has long been appreciated that motility organelles of *P. aeruginosa* are recognized by mammalian innate immune sensors. The extracellular Toll-like receptor (TLR) 5 and intracellular Naip5 protein both bind flagellin and activate signalling pathways upstream of NF- κ B activation and NLRC4 inflammasome activation, respectively [25,26]. The additional ability to sense and respond to flagellar activity has recently been described by Berwin and colleagues [27–29]. *P. aeruginosa* strains lacking flagellar motor stator complexes (*motAB motCD*) assemble a non-motile flagellum and are ca. 100-fold more resistant to phagocytosis by neutrophils and macrophages than motile bacteria. Phagocytic cells respond to flagellar motility by activating the PI3K/AKT pathway via a TLR5-independent mechanism, leading to actin-dependent bacterial engulfment [30].

P. aeruginosa also elicit signaling responses in epithelial cells that are dependent on the flagellum [31]. Binding of bacterial aggregates to the apical surface of polarized epithelial monolayers results in the formation of a protrusion enriched in the lipid PIP₃, and polarity and adherens junction proteins such as Par3, aPKC, Rac1 and Par6 α . These protrusions do not form under aflagellate mutants, although the requirement for a motile flagellum was not specifically tested in this study. Protrusion formation is necessary for subsequent activation of NF- κ B, which also requires bacterial expression of the Type 3 secretion system “injectisome”.

Infection models suggest that Type IV pili, as well as flagella, are sensed during mammalian infection. A murine model, in which gastrointestinal colonization by *P. aeruginosa* is followed by systemic dissemination when neutropenia is experimentally induced, was recently used to examine bacterial proteins required for *in vivo* “fitness” [32]. The technique of insertion sequencing (INSeq) was used to measure the frequency of individual transposon (Tn)-insertion mutants in the infecting inoculum, at the cecal mucosa, and in the spleen. Tn insertions in genes encoding components of the flagellar system were overrepresented in bacteria that colonized the cecum. This result was attributed to recognition of flagellin by multiple innate immune sensors. Tn insertions in most Type IVa pili assembly genes—but not insertions in other surface adhesins such as Cup fimbriae or Type IVb pilins—also

increased fitness for cecal colonization. As disruption of the retraction ATPases PilT and PilU, which results in increased surface expression of Type IVa pili, was associated with decreased fitness, these findings suggested that Type IVa pili might also elicit pro-inflammatory host responses that lead to increased bacterial clearance. An earlier report describing activation of caspase-1 in mouse bone-marrow derived macrophages transfected with *P. aeruginosa* pilin is consistent with this hypothesis, although a host sensor for pilin was not identified in this study [33]. A human clinical study examining correlations between virulence factor expression and *P. aeruginosa* acute infection has documented a positive association between flagellar motility and symptomatic infection in patients with urinary tract infections [34]. Whether this association reflects increased inflammatory responses to flagellated bacteria, or an advantage of flagellated bacteria in colonizing or ascending the urinary tract catheters frequently found in these patients cannot be ascertained from this observational study.

Conclusion

The past few years have literally given us a new view on *P. aeruginosa* motility, as particle-tracking algorithms have allowed single bacterial cells to be followed as they arrive at, explore and colonize experimental surfaces. The heterogeneity of bacterial behaviors documented by these studies begs the question of how such variation is achieved, with one paradigm—asymmetric inheritance of enzymes that regulate c-di-GMP levels—receiving experimental support. Recent work also highlights T4P attachment and retraction as signals for cAMP production by surface-associated bacteria, a signaling circuit that would reinforce T4P driven motility and perhaps favor transitions to surface associated virulence, c-di-GMP production and ultimately biofilm formation. These “pro-virulence” aspects of motility are balanced by *in vivo* work that illustrates the costs to being motile: recognition by multiple innate immune systems that respond to motility structures and functions. A future challenge will be to understand how all of these inputs sum during infection, especially if we wish to tip the balance in favor of the host.

Acknowledgements

Research in the Kazmierczak laboratory has been funded by the National Institutes of Health (Grants AI075051 and AI081825) and Burroughs Wellcome Fund (Investigator in the Pathogenesis of Infectious Disease Award).

References and recommended reading

1. Caiazza NC, O'Toole GA. SadB is required for the transition from reversible to irreversible attachment during biofilm formation by *Pseudomonas aeruginosa* PA14. *J Bacteriol.* 2004; 186:4476–4485. [PubMed: 15231779]
2. Cowles KN, Gitai Z. Surface association and the MreB cytoskeleton regulate pilus production, localization and function in *Pseudomonas aeruginosa*. *Mol Microbiol.* 2010; 76:1411–1426. [PubMed: 20398206]
3. Guvener ZT, Harwood CS. Subcellular location characteristics of the *Pseudomonas aeruginosa* GGDEF protein, WspR, indicate that it produces cyclic-di-GMP in response to growth on surfaces. *Molecular Microbiology.* 2007; 66:1459–1473. [PubMed: 18028314]
4. Irie Y, Borlee BR, O'Connor JR, Hill PJ, Harwood CS, Wozniak DJ, Parsek MR. Self-produced exopolysaccharide is a signal that stimulates biofilm formation in *Pseudomonas aeruginosa*. *Proc Natl Acad Sci U S A.* 2012; 109:20632–20636. [PubMed: 23175784]

5. Conrad JC, Gibiansky ML, Jin F, Gordon VD, Motto DA, Mathewson MA, Stopka WG, Zelasko DC, Shrout JD, Wong GC. Flagella and pili-mediated near-surface single-cell motility mechanisms in *P. aeruginosa*. *Biophys J*. 2011; 100:1608–1616. [PubMed: 21463573]
6. Bruzard J, Tarrade J, Coudreuse A, Canette A, Herry JM, Taffin de Givenchy E, Darmanin T, Guittard F, Guilbaud M, Bellon-Fontaine MN. Flagella but not type IV pili are involved in the initial adhesion of *Pseudomonas aeruginosa* PAO1 to hydrophobic or superhydrophobic surfaces. *Colloids Surf B Biointerfaces*. 2015; 131:59–66. [PubMed: 25950497]
7. Kulasekara BR, Kamischke C, Kulasekara HD, Christen M, Wiggins PA, Miller SI. c-di-GMP heterogeneity is generated by the chemotaxis machinery to regulate flagellar motility. *Elife*. 2013; 2:e01402. [PubMed: 24347546] ** This paper uses a novel c-di-GMP biosensor to show that asymmetric distribution of a phosphodiesterase generates variation in intracellular c-di-GMP levels and heterogeneous motility behavior in postdivision cells.
8. Paul K, Nieto V, Carlquist WC, Blair DF, Harshey RM. The c-di-GMP binding protein YcgR controls flagellar motor direction and speed to affect chemotaxis by a “backstop brake” mechanism. *Mol Cell*. 2010; 38:128–139. [PubMed: 20346719]
9. Boehm A, Kaiser M, Li H, Spangler C, Kasper CA, Ackermann M, Kaever V, Sourjik V, Roth V, Jenal U. Second messenger-mediated adjustment of bacterial swimming velocity. *Cell*. 2010; 141:107–116. [PubMed: 20303158]
10. Li G, Brown PJ, Tang JX, Xu J, Quardokus EM, Fuqua C, Brun YV. Surface contact stimulates the just-in-time deployment of bacterial adhesins. *Mol Microbiol*. 2012; 83:41–51. [PubMed: 22053824]
11. Cairns LS, Marlow VL, Bissett E, Ostrowski A, Stanley-Wall NR. A mechanical signal transmitted by the flagellum controls signalling in *Bacillus subtilis*. *Mol Microbiol*. 2013; 90:6–21. [PubMed: 23888912]
12. Caiazza NC, Merritt JH, Brothers KM, O'Toole GA. Inverse regulation of biofilm formation and swarming motility by *Pseudomonas aeruginosa* PA14. *J Bacteriol*. 2007; 189:3603–3612. [PubMed: 17337585]
13. Anyan ME, Amiri A, Harvey CW, Tierra G, Morales-Soto N, Driscoll CM, Alber MS, Shrout JD. Type IV pili interactions promote intercellular association and moderate swarming of *Pseudomonas aeruginosa*. *Proc Natl Acad Sci U S A*. 2014; 111:18013–18018. [PubMed: 25468980]
14. Kato J, Kim H-E, Takiguchi N, Kuroda A, Ohtake H. *Pseudomonas aeruginosa* as a model microorganism for investigation of chemotactic behaviors in ecosystem. *J Bioscience Bioengineering*. 2008; 106:1–7.
15. Jin F, Conrad JC, Gibiansky ML, Wong GCL. Bacteria use type-IV pili to slingshot on surfaces. *Proc Natl Acad Sci U S A*. 2011; 108:12617–12622. [PubMed: 21768344]
16. Zhang R, Ni L, Jin Z, Li J, Jin F. Bacteria slingshot more on soft surfaces. *Nat Commun*. 2014; 5:5541. [PubMed: 25412641]
17. Leighton, TL.; Buensuceso, RN.; Howell, PL.; Burrows, LL. *Environ Microbiol*. 2015. Biogenesis of *Pseudomonas aeruginosa* type IV pili and regulation of their function.
18. Persat A, Inclan YF, Engel JN, Stone HA, Gitai Z. Type IV pili mechanochemically regulate virulence factors in *Pseudomonas aeruginosa*. *Proc Natl Acad Sci U S A*. 2015* This paper uses a cAMP-responsive transcriptional reporter to demonstrate that Type IV pilus retraction is required for increased cAMP levels in surface attached bacteria, and proposes that a MCP-like protein of the Pil/Chp chemotaxis cluster mediates this response.
19. Beaussart A, Baker AE, Kuchma SL, El-Kirat-Chatel S, O'Toole GA, Dufrene YF. Nanoscale Adhesion Forces of *Pseudomonas aeruginosa* Type IV Pili. *ACS Nano*. 2014; 8:10723–10733. [PubMed: 25286300]
20. Luo Y, Zhao K, Baker AE, Kuchma SL, Coggan KA, Wolfgang MC, Wong GC, O'Toole GA. A hierarchical cascade of second messengers regulates *Pseudomonas aeruginosa* surface behaviors. *MBio*. 2015; 6:e02456–02414. [PubMed: 25626906]
21. Kuchma SL, Ballok AE, Merritt JH, Hammond JH, Lu W, Rabinowitz JD, O'Toole GA. Cyclic-di-GMP-mediated repression of swarming motility by *Pseudomonas aeruginosa*: the *pilY1* gene and

- its impact on surface-associated behaviors. *J Bacteriol.* 2010; 192:2950–2964. [PubMed: 20233936]
22. Zhao K, Tseng BS, Beckerman B, Jin F, Gibiansky ML, Harrison JJ, Luijten E, Parsek MR, Wong GC. Psl trails guide exploration and microcolony formation in *Pseudomonas aeruginosa* biofilms. *Nature.* 2013; 497:388–391. [PubMed: 23657259] ** This paper uses particle-tracking algorithms to follow Type IV pilus mediated movement of bacteria along self-deposited trails of the exopolysaccharide Psl, and proposes a power law model to explain heterogeneous surface colonization and microcolony formation.
 23. Siryaporn A, Kim MK, Shen Y, Stone HA, Gitai Z. Colonization, competition, and dispersal of pathogens in fluid flow networks. *Curr Biol.* 2015; 25:1201–1207. [PubMed: 25843031]
 24. Siryaporn A, Kuchma SL, O'Toole GA, Gitai Z. Surface attachment induces *Pseudomonas aeruginosa* virulence. *Proc Natl Acad Sci U S A.* 2014; 111:16860–16865. [PubMed: 25385640] * This paper demonstrates differing levels of virulence toward amoeba by genetically identical *P. aeruginosa* grown in liquid versus at a surface. The authors use genetic evidence to propose a two signal model (surface attachment and cell density) to explain host-nonspecific induction of virulence.
 25. Vijay-Kumar M, Gewirtz AT. Flagellin: key target of mucosal innate immunity. *Mucosal Immunol.* 2009; 2:197–205. [PubMed: 19242410]
 26. Vance RE. The NAIP/NLRC4 inflammasomes. *Curr Opin Immunol.* 2015; 32:84–89. [PubMed: 25621709]
 27. Amiel E, Lovewell RR, O'Toole GA, Hogan DA, Berwin B. *Pseudomonas aeruginosa* evasion of phagocytosis is mediated by loss of swimming motility and is independent of flagellum expression. *Infect Immun.* 2010; 78:2937–2945. [PubMed: 20457788]
 28. Patankar YR, Lovewell RR, Poynter ME, Jyot J, Kazmierczak BI, Berwin B. Flagellar motility is a key determinant of the magnitude of the inflammasome response to *Pseudomonas aeruginosa*. *Infect Immun.* 2013; 81:2043–2052. [PubMed: 23529619]
 29. Lovewell RR, Collins RM, Acker JL, O'Toole GA, Wargo MJ, Berwin B. Step-wise loss of bacterial flagellar torsion confers progressive phagocytic evasion. *PLoS Pathog.* 2011; 7:e1002253. [PubMed: 21949654]
 30. Lovewell RR, Hayes SM, O'Toole GA, Berwin B. *Pseudomonas aeruginosa* flagellar motility activates the phagocyte PI3K/Akt pathway to induce phagocytic engulfment. *Am J Physiol Lung Cell Mol Physiol.* 2014; 306:L698–707. [PubMed: 24487390]
 31. Tran CS, Eran Y, Ruch TR, Bryant DM, Datta A, Brakeman P, Kierbel A, Wittmann T, Metzger RJ, Mostov KE, et al. Host cell polarity proteins participate in innate immunity to *Pseudomonas aeruginosa* infection. *Cell Host Microbe.* 2014; 15:636–643. [PubMed: 24832456]
 32. Skurnik D, Roux D, Aschard H, Cattoir V, Yoder-Himes D, Lory S, Pier GB. A comprehensive analysis of in vitro and in vivo genetic fitness of *Pseudomonas aeruginosa* using high-throughput sequencing of transposon libraries. *PLoS Pathog.* 2013; 9:e1003582. [PubMed: 24039572] * This paper uses insertion sequencing (INSeq) to show that *P. aeruginosa* expression of flagella and type IV pili is associated with decreased fitness in a mouse model of colonization and dissemination, and suggests that immune responses toward these motility structures impose this negative selection.
 33. Arlehamn CS, Evans TJ. *Pseudomonas aeruginosa* pilin activates the inflammasome. *Cell Microbiol.* 2011; 13:388–401. [PubMed: 20955240]
 34. Ledizet M, Murray TS, Puttagunta S, Slade MD, Quagliarello VJ, Kazmierczak BI. The ability of virulence factor expression by *Pseudomonas aeruginosa* to predict clinical disease in hospitalized patients. *PLoS ONE.* 2012; 7:e49578. [PubMed: 23152923]

Highlights

Particle-tracking algorithms show a wide range of *P. aeruginosa* motility at surfaces.

Asymmetry of c-di-GMP levels in cells correlates with varied motility post-division.

Retraction of attached Type IV pili is a signal for increased cAMP production.

Surface attachment and cell density are independent signals for increased virulence.

Innate immunity distinguishes motile and nonmotile flagella and reacts to the former.

Figure 1. Twitching motility allows *P. aeruginosa* to move “up” and “across” fluid stream lines *P. aeruginosa* (blue) seeded into a branched microfluidic network uses T4P-mediated motility to move “upstream” and to cross into side branches of the network. Swimming bacteria that cannot twitch, like *Proteus mirabilis* (red) outcompete *P. aeruginosa* in the seeded branch, but cannot gain access to side branches. (N.D. = not detected).