The Force Awakens: the dark side of mechanosensing in bacterial pathogens

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Abstract

For many bacteria, the ability to sense physical stimuli such as contact with a surface or a potential host cell is vital for survival and proliferation. This ability, and subsequent attachment, confers a wide range of benefits to bacteria and many species have evolved to take advantage of this. Despite the impressive diversity of bacterial pathogens and their virulence factors, mechanosensory mechanisms are often conserved. These include sensing impedance of flagellar rotation and resistance to type IV pili retraction. There are additional mechanisms that rely on the use of specific membrane-bound adhesins to sense either surface proximity or shear forces. This review aims to examine these mechanosensors, and how they are used by pathogenic bacteria to sense physical features in their environment. We will explore how these sensors generate and transmit signals which can trigger modulation of virulence-associated gene expression in some of the most common bacterial pathogens: *Pseudomonas aeruginosa*, *Proteus mirabilis*, *Escherichia coli* and *Vibrio* species.

Introduction

Most bacteria live in communities that are associated with a surface, and attachment has a wide range of benefits to the survival and spread of many species [1], [2]. It allows bacteria access to nutrients which settle or adsorb on surfaces in higher concentrations than the surrounding environment, allowing bacteria growth in low nutrient environments [3], [4]. This makes surface sensing and

attachment extremely important to both pathogenic and non-pathogenic bacteria. For pathogens within a host organism, attachment provides resistance to removal by fluid flow, while close contact to host cells ensures access to the host's resources and enables injection of virulence factors [5]–[7]. Adhesion to a surface helps protect against antibiotics by reducing the net negative charge on the bacterial cells [8] and by enhancing the stability of the plasma membrane [9]. Attachment also allows for localised increases in bacterial cell concentration and for specialization of cells and other cooperative behaviours within that community [10].

Adhesion is the initial stage in the development of biofilms, bacterial communities suspended in an extracellular matrix. This matrix is composed of a mixture of polysaccharides, proteins and extracellular DNA to form a three-dimensional structure [11]. Biofilms are manufactured by bacteria to improve their survival and proliferation by conferring a series of benefits such as improved protection from mechanical removal (shear force of fluid flow or abrasion) and attack by chemical (such as antibiotics) and biological agents (such as phage or predators). Bacterial attachment can however be seriously detrimental to human interests, health in the case of pathogens or industrial output in the case of biofouling organisms attached to industrial surfaces [12], [13].

Bacteria can respond to a wide range of environmental cues such as nutritional gradients [14], osmolarity [15], [16] and the presence of host molecules [17]. They feed this information into complex regulatory pathways that control gene expression. In addition to chemical signals, bacteria can respond to physical cues in their environment. The ability to perceive when they are in contact with a surface is vital for the transition from a free-living planktonic lifestyle to a sedentary one comprising adhesion to a surface. This is an even more important consideration when bacteria need to survive within a host. Accordingly, the numerous environmental niches present within a host often require pathogens to use special tools to survive and prosper. These tools, or virulence factors, are highly diverse in their forms and functions.

Following the sensing of surface attachment, or indeed appropriate niche-specific metabolic cues, alteration in protein expression is facilitated through modulation of gene expression by transcription factors (TFs) [18]. Bacteria encode hundreds of diverse TFs to cope with the immense diversity of environmental signals that require modulation of gene expression [19]. These predominantly take the form of either two-component sensor kinase regulators that operate phospho-relay signal transduction pathways [20], or cytosolic TFs such as LysR and AraC type transcriptional regulators [20], [21]. Typically, a specific helix turn helix motif within the DNA-binding domain of TFs facilitate direct interaction with a consensus sequence upstream of the coding region of the target genes [21]. Through modulation of DNA topology and interaction with RNA polymerase and other TFs, expression levels of the target genes can be manipulated [22]. In the case of cytosolic TFs, further selectivity and specificity is conferred by the binding of small molecules that are often niche-specific metabolites and serve as cofactors for TF activity [23].

It is important to understand the interaction between bacterial surface sensing systems and the regulation of virulence factors in order to develop a complete picture of pathogen behaviour within the host. Improved understanding of this has the potential to improve treatments of numerous infections and ultimately improve patient outcomes. Many of the mechanisms by which bacteria attach to surfaces, both living and inanimate, are relatively well studied [24]–[26]. Furthermore, the study of how proximity to surfaces is sensed and interpreted via signal transduction to allow for the appropriate expression of virulence factors is a rapidly evolving area with considerable recent advancements. In this review, we will discuss the colonisation factors mediating these attachment events, the signal transduction pathways that allow for interpretation of the surface signal, the regulatory proteins that mediate a change in virulence factor expression and the specific virulence factors reported to be regulated by these processes in specific pathogenic bacteria.

Non-pathogenic mechanosensing

While this review focuses on the mechanosory mechanisms of pathogenic bacteria it is important to highlight that these and other mechanisms are not limited to pathogens. One of the best studied examples is *Caulibacter crescentus* which possesses a tight adherence (Tad) pilus that shares a number of common features to the type IV pilus. Ellison et al. observed that the Tad pilus appeared to perform a similar mechanosenory function to the type IV pilus, with the Tad pilus undergoing cyclical extension and retraction [27]. Upon contact with a surface the Tad pilus attaches resulting in resistance to pilus retraction. After attachment extension and retraction cycles stopped and synthesis of the holdfast adhesin (an important factor in the attachment process) increased. It is thought that retraction of the Tad pilus is used as a mechanosensory mechanism to initiate changes in attachment related gene expression allowing *C. crescentus* to react dynamically to its physical environment [27]. This and other examples of mechanosensing in non pathogens have recently been covered, hence the focus of this review are specific mechanisms used by pathogens [28].

Colonisation factors with roles in surface sensing

Flagella

The sensing of physical cues by mechanical sensory systems forms an important part of environmental recognition. These mechanosensors allow bacteria to respond to shear forces exerted by flow [29], [30], gravity [31] and the physical interaction with surfaces and other objects in their environment [32]. For pathogenic bacteria, the sensing of physical interactions with surfaces is vital for their survival and success within the host. Flagella are whip-like organelles, ranging from 1-9 µm in length [33], anchored to the bacterial cell surface and used primarily for cell motility [34]. Despite their central role in motility, flagella of many bacterial species have been shown to play roles in surface sensing, adhesion and biofilm formation [32], [34]–[36]. Bacterial surface sensing can be mediated by the interference in the rotation of the bacterial flagella by a proximal surface [37]. This interference is often termed flagella impedance. Flagella have been observed to function as mechanosensors capable

of detecting surface contact in several organisms including *Vibrio parahaemolyticus* [38], *P. aeruginosa* [39] and *Bacillus subtilis* [40].

Type IV pili

The use of motility organelles as mechanosensors does not appear to be unique to flagella. Type IV pili are long (1-4 μm), thin (50–80 Å) [41], membrane-bound filamentous structures utilised by a wide range of organisms for a variety of functional purposes including roles in motility and attachment [42]–[44]. They are extended via polymerisation by an assembly ATPase, can attach to a surface and retract via depolymerisation powered by a retraction ATPase [45]. This gives rise to what is known as twitching motility [46] and allows bacteria that employ it to spread swiftly across surfaces [47]–[49]. Twitching motility has been observed in several bacterial species and is particularly well studied in *P. aeruginosa* [50]. While acting as virulence factors in their own right, like flagella, type IV pili play an important role as mechanosensors.

Molecular mechanisms of surface sensing and virulence regulation in bacterial pathogens

Pseudomonas aeruginosa

P. aeruginosa is an opportunistic pathogen casuing both chronic and acute infections in a wide range of hosts and at many different sites within the body. In humans, acute disease caused by *P. aeruginosa* is often characterised by cytotoxicity, invasion of host tissue and can progress to systemic infection and sepsis. This pattern of pathogenesis is the results from expression of numerous proteases, toxins and type III secretion system (a needle-like complex that allows injection of specific effectors into a host cell [51], [52]) activity. This can be manifested by infection of burn injuries on the skin and ventilator-associated pneumonia. Chronic infection on the other hand is noncytotoxic, far less invasive and does not typically lead to systemic infection. This pattern of pathogenesis is commonly seen in patients with cystic fibrosis were *P. aeruginosa* forms a biofilm that cannot be cleared and results in

damaging long-term inflammation in the lungs [53]–[56]. The diversity of these infections is orchestrated by accurate sensing of the environment and the production of an appropriate virulence response.

The polar flagellum of *P. aeruginosa* has been shown to play a central role in the attachment process as a part of a mechanosensory system. *P. aeruginosa* cells have been observed to initially attach to a surface, often only temporarily, with many cells quickly detaching. However, those that remain attached, bind irreversibly to the surface rapidly halting the rotation of their polar flagella [57], [58]. Impedance of rotation occurs via the interaction of the signal recognition particle (SRP)-type guanosine triphosphatase (GTPase) FlhF with FliG (a component of the flagellar motor machinery) and FimV, (a peptidoglycan binding protein which forms part of the structural complex of the *P. aeruginosa* type IV pilus and has been shown to increase the production of cyclic adenosine monophosphate [cAMP]) [39]. This interaction triggers an increase in intracellular cAMP which activates further downstream regulatory events, altering flagellar rotation [39]. FlhF is a known regulator of flagellar positioning, is conserved between other bacterial species with polar flagella [59] and, when deleted, results in flagella being positioned in areas other than the cell pole [60]. Schniederberend et al. [39] found that the flagella of *P. aeruginosa* Δ*flhf* mutants continued to spin even after flagellar attachment but could have the wild-type phenotype restored by 23 independent suppressor mutations which were mapped to the cAMP-dependent transcriptional regulator Vfr.

Vfr has been shown to control the expression of over 200 *P. aeruginosa* virulence genes [61], [62]. These Vfr regulated genes include surface localised virulence factors such as the type II and type III secretion systems [61] (T2SS and T3SS), the former of which is used by *P. aeruginosa* to transport virulence molecules such as proteases, the latter can inject specific effectors that modulate their eukaryotic hosts to promote bacterial survival and proliferation [51], [52]. Additionally, Vfr positively regulates the production of proteases, toxins and the type IV pilus, a motility organelle and major surface adhesin [61], [63]–[65], while downregulating flagellar genes [66]. These virulence factors are

primarily associated with acute *P. aeruginosa* infection phenotypes as opposed to the chronic infection phenotype commonly seen in the lunges of cystic fibrosis (CF) patients [67].

In addition to the flagella, *P. aeruginosa* can sense contact with surfaces using their type IV pili [29], [68], [69]. Persat et al. [69] and Inclan et al. [70] demonstrated that once type IV pili have attached, a signal is generated when the organelle attempts to retract. This resistance to retraction triggers the Pil-Chp chemosensory system, leading to increased production of cAMP which in turn activates the expression of virulence-related genes. This signal transduction happens via an accessory protein FimL, which is part of the Pil-Chp system, interacting with FimV. As mentioned earlier FimV is a type IV structural protein shown to increase the production of cAMP. It is thought that FimL, along with PilG, localises at the cell poles via FimV. This allows PilG to be phosphorylated, activating the Pil-Chp system which regulates the membrane-bound adenylate cyclase CyaB [71], [72]. CyaB is a major source of cAMP which, similar to the mechanosensing pathway activated by the flagella, binds to the Vfr virulence regulator and increases expression of proteins associated with acute virulence [61]. The surface sensing signal generated by the type IV pili in *P. aeruginosa* is relatively short-lived but results in further promotion of type IV pilus activity, giving rise to a positive feedback loop which promotes a stronger signal for activation of virulence and biofilm pathways [69]. Phylogenetic comparison between *P. aeruginosa* and other gamma-proteobacteria show that the components of this system (FimL and FimV) are conserved in species that encode type IV pili and a Chp-like sensory system, suggesting that this mechanosensory mechanism might be a common approach utilised in other organisms of that group like *Acinetobacter baumannii*, *Vibrio cholerae* and *Vibrio vulnificus* [70].

In addition to flagellar impedance and interference with type IV pili retraction, *P. aeruginosa* is also thought to possess a third surface sensing mechanism which acts upon contact with a host cell surface. This system is thought to utilise the product of the *pilY1* gene, which encodes a putative mechanosensory protein and adhesin [73] that is a stimulator of virulence and biofilm formation in response to surface attachment. PilY1 is normally expressed on the cell surface and upregulated

during surface contact and attachment [74]. Siryporn et al. [74] found that Δ*pilY1* mutants and mutants in genes encoding minor pilin proteins PilW and PilX showed poor virulence phenotypes in a eukaryotic amaeboa infection model (*Dictyostelium discoideum*). This effect was shown to be independent of the loss of the type IV pilus as mutants in genes encoding other pilin proteins, such as *ΔpilB* and *ΔpilC*, which failed to produce a functional type IV pilus filament still retained their virulence phenotypes. Virulence modulation through PilY1, PilW and PilX took place despite a weakened attachment phenotype in mutants that lacked the ability to produce type IV pili and appears to operate separately to the other functions of the pilus filament. The Pil-Chp pathway also involves the physical interaction of PilJ and FimS in response to surface-associated signals. FimS acts together with AlgR as a two-component system and is a transmembrane sensor kinase [75]. The FimS-AlgR system (its own expression promoted by cAMP-Vfr) upregulates *pilY1* gene expression. PilY1 along with the PilVWX minor pilins localize at the inner cell membrane and act via FimS-AlgR, PilJ and Vfr to autoregulate their expression [68]. PilY1 contains a mechanosensing Von Willebrand factor A domain similar to those produced by human endothelial cells to facilitate platelet aggregation [76]. This domain is stretched when exposed to shear force [77] and mutation can result in constitutive activation of *P. aeruginosa* virulence without surface attachment [74]. It is thought that PilY1 signals through the type IV pilus PilMNOP alignment complex and the diguanylate cyclase SadC, both localised at the inner membrane [68], [78]. This activates cyclic dimeric GMP (c-di-GMP) production and in addition to the aforementioned virulence against amoeba has been shown to influence swarming motility and early-stage biofilm formation [79]. In *P. aeruginosa* this surface sensing mechanism is important for attachment, colonisation and appropriate triggering of virulence in several host organisms, ranging from mammalian models to single-celled amoebae [80], [81].

The chemotaxis-like Wsp system has also been implicated in surface sensing. Signals are generated by growth on a surface and thought to be recognized by the membrane bound receptor WspA. While the exact nature of the signal remains unknown, it is believed to be the result of physical changes to the periplasmic environment, caused by either the mechanical strain of surface contact or the

physiological changes that accompany the transition to a biofilm state. This signal is transitted via the other components of the Wsp system (WspBCDEFR), present in the cell cytoplasm, resulting in phosphorylation of WspR. Phosphorlyated WspR then synthesises c-di-GMP, activating biofilm formation, virulence gene expression and supressing motility [82].

Surface contact-induced virulence activation has been further explored by assaying virulence against *D. discoideum* following attachment of bacteria to a variety of surfaces of varying composition, including plastics, glass, polyacrylamide and agar [74]. It was found that the activation of virulence was similar on all surfaces and therefore appeared to require the physically rigid surface rather than any specific chemical surface composition. Siryaporn et al. [74] hypothesize that non-specific virulence activation, could allow *P. aeruginosa* to infect a wide range of host species despite differences in their individual surface receptors and tissue chemistries. Similar results were observed in mouse macrophages to those seen in *D. discoideum*, with surface-attached *P. aeruginosa* shown to be more virulent than planktonic bacteria as measured host cells lysis. Siryaporn et al. [74] also found that when bacteria were allowed to attach to the surface of the plant species *Epipremnum aureum* and grown in the presence of *D. discoideum* it resulted in secretion of virulence factors toxic to *D. discoideum*, further suggesting activation of virulence in response to non-specific surface attachment.

It has been proposed that regulation of surface sensing is integrated into other regulatory networks such as quorum sensing. Siryaporn et al. [74] grew bacteria to different growth phases, allowed them to attach to surfaces, exposed them to *D. discoideum* and measured host killing. Virulence was drastically increased between mid and late exponential phase. This suggested that increased cell density could be activating quorum sensing and in turn affecting virulence factor expression. Quorum sensing in *P. aeruginosa* is primarily regulated by the transcriptional activator LasR [83]. Siryaporn et al. [74] observed that *lasR* mutants showed a disrupted virulence phenotype. However, neither high-density planktonic *P. aeruginosa* cells nor over-expression of LasR quorum sensing in planktonic bacteria resulted in lysis of eukaryotic cells. From this the authors conclude that quorum sensing alone

is not sufficient for optimum virulence and that mechanosensing is also of crucial importance. As such, this indicates that PilY1 mechanosensing and LasR dependent quorum sensing act in concert to ensure appropriate virulence regulation in *P. aeruginosa*. This non-specific mechanosensing behaviour is believed to allow *P. aeruginosa* to infect a broad range of host species while maintaining carefully regulated virulence mechanisms.

Proteus mirabilis

Proteus mirabilis is a pathogen associated with infections of the urinary tract. On contact with a surface *P. mirabilis* can undergo a phenotypic change from small swimmer cells, adapted to life in liquid, into longer swarmer cells expressing high numbers of flagella that promote persistence within the host [84]–[86]. This differentiation is a metabolically costly process and involves the expression of 50 genes, including several known to encode virulence factors which improve *P. mirabilis* survival and mediate damage to the host [87].

It has been shown that, like *P. aeruginosa*, *P. mirabilis* uses flagellar impedance as a signal of surface contact [88]. Inhibition of the rotation of the flagellar motor can result from several different physical interactions. These include contact with solid surfaces, high viscosity liquids and the binding of flagellar components by specific antibodies [89]. Belas et al. [89] found that mutations in many flagellar genes resulted in *P. mirabilis* losing the ability to switch to a swarming phenotype. Surprisingly, it was also found that mutation of genes that encode for the flagellar basal structure (*fliF*, *fliG* and *fliL*) resulted in differentiation into swarmer-like cells in non-inducing liquid media and hyper-elongated swarmer cells when grown on solid agar. The constitutive activation of this swarmer differentiation pathway via specific flagellar components further reinforces the role of the flagella as a mechanosensory organelle capable of detecting proximity to a surface [90]. Activation of the flagellar sensory pathway via these mutations was shown to up-regulate the expression of *zapA* which encodes a metalloprotease, an enzyme that can degrade antibacterial peptides, such as LL-37 and β-defensin,

248 that are used by the immune system to help fight bacterial infection [87], [91]. Sensory pathway
249 activation was also shown to up-regulate the expression of *hpmB* [89], the first gene in the *hpmBA*
250 operon. This operon also encodes the HpmA hemolysin which is a major cytotoxin used by many
251 clinical *P. mirabilis* strains to cause tissue damage and facilitate cellular invasion by the bacteria [87].
252 Mechanosensing of surfaces via the flagella allows *P. mirabilis* to carefully regulate the differentiation
253 process from swimmer to swarmer cells and so to more efficiently regulate the production of virulence
254 factors needed for colonisation and persistence within its host.

255

256 ***Escherichia coli***

257 *Escherichia coli* are an extremely diverse species of commensal and pathogenic bacteria found in the
258 GI tract of many organisms. Many *E. coli* strains are not harmful to their hosts, but certain strains can
259 cause infections at various sites in the body including infections of the urinary and GI tract [92]. In
260 addition to using numerous chemical sensory systems, *E. coli* has also been shown to regulate its
261 virulence through the use of mechanosensors [30].

262 *Enterohemorrhagic E. coli* (EHEC) strain O157:H7 is a problematic pathogen that causes haemorrhagic
263 colitis, and in more severe cases kidney damage in the form of haemolytic uremic syndrome (HUS).
264 The development of HUS is linked with high morbidity and mortality [93]. EHEC infections are typically
265 acquired by ingestion of foods contaminated by faecal material. EHEC uses a T3SS, several adhesins
266 and Shiga toxins as virulence factors to mediate disease [94]–[96]. The T3SS is encoded on a
267 horizontally acquired pathogenicity island, designated as the locus of enterocyte effacement (LEE) and
268 consists of 4 polycistronic operons (LEE1-5), activated by the first of these (LEE1). LEE1 contains a
269 crtically important activator of the LEE,known as Ler, or LEE-encoded regulator [97]. The T3SS allows
270 EHEC to inject effector proteins into the host cells that line the large intestine, remodelling the actin
271 cytoskeleton to form pedestals on which the bacteria adhere tightly. These pedestals are known as
272 attaching and effacing (A/E) lesions, are highly characteristic of EHEC infection and are very effective

at promoting their persistence in the intestinal environment [98], [99]. Expression of the LEE in EHEC is controlled by both chemical signals and physical forces acting on mechanosensory apparatus. The LEE has been shown to be induced by several environmental cues including temperature shifts as the bacteria travels through the gastrointestinal (GI) tract, neutral pH after exit from the stomach, reduced oxygen concentrations, quorum sensing molecules, bicarbonate, glucose and hormone signals from the host [100]–[104].

Alsharif et al. [30] showed that the LEE is only weakly induced by chemical environmental signals alone. Alsharif et al. [30] infected HeLa epithelial cells with EHEC and monitored LEE1 promoter activity using fluorescence microscopy and GFP producing reporter strains. Bacteria that attached to host cells displayed strong activation of *ler* and formation of the characteristic A/E lesions. However, bacterial cells that attached to glass rather than the host cell surface showed comparatively weak LEE activation even in Dulbecco's Modified Eagle Medium (DMEM) which has been previously shown to be an activator of Ler [105]. *Ler* is activated by GrlA (itself encoded on the LEE pathogenicity island) and in *ΔgrlA* strains LEE1 was not strongly induced on host-attached nor unattached bacteria and significantly reduced formation of A/E lesions was observed. A recent paper by Sirisaengtaksin et al. [106] has shown that GrlA is also required to transition from the inner membrane of planktonic cells to the cytoplasm to efficiently activate virulence genes. Bacteria expressing GrlA-GFP fusion proteins were adhered to polylysine coated microfluidic channels before applying fluid shear. GrlA is a membrane-bound transcription factor under normal conditions but when shear forces are encountered by EHEC, GrlA is released into the cytoplasm. Here it binds and activates the LEE1 promoter triggering the expression of the LEE operon [106] and hence optimal T3SS activity.

These studies illustrate how *E. coli* senses changes in physical force and alters the expression of virulence factors in response (LEE operon genes). Despite the specific attachment factor responsible for binding to the polylysine coated substrate not being reported by Sirisaengtaksin et al. [106], the

297 flagellum of pathogenic *E. coli* have been shown to play a role in adhesion [107] and more recently in
298 mechanosensing [108].

299 Laganenka et al. [108] show that the flagella of various pathogenic *E. coli* act as mechanosensors
300 during infection, aiding in the regulation of motility to improve survival within the host. Laganenka et
301 al. [108] compared motility of a variety of pathogenic and commensal *E. coli* strains by soft agar
302 motility assays and single-bacterium movement tracking. Increased motility was observed in
303 pathogenic strains when grown in low percentage agar plates compared to liquid media. This was not
304 the case in commensal strains, which showed similar motility in both media forms. To examine the
305 underlying regulatory mechanism, changes in flagellar gene expression were monitored using GFP
306 fluorescence reporters. This revealed that in pathogenic strains *fliA* (encoding the sigma factor
307 controlling flagellar genes) and *fliC* (encoding flagellin, the major flagellar structural protein) [109]
308 were highly downregulated in liquid culture [108].

309 Laganenka et al. [108] hypothesize that increased flagellar impedance due to the porous structure of
310 the low percentage agar compared to liquid media results in an increased physical load on the flagellar
311 motor. *E. coli* flagella have been shown to respond to flagellar motor load by the recruitment of
312 additional stators (the force generation units used to drive motor motion) [110]. Knockout mutants of
313 the *fliC* and *motA* genes, which encode key components of the flagellar filament and the motor-stator
314 complex respectively, were constructed. These mutants had either lower physical loads on the motor
315 (*ΔfliC*) or non-functional motors (*ΔmotA*). Both modifications resulted in pathogenic strains that were
316 unable to activate motility in either liquid or agar media. As previously discussed in this review,
317 impedance of the flagella can also take place due to attachment or contact with a surface [46], [111].
318 Laganenka et al. [108] studied the effects of increasing flagellar load by forced surface contact during
319 centrifugation and by growth on semi-solid agar, with both conditions resulting in increased
320 expression of *fliC*.

These observations indicate that upregulation of flagellar genes and the associated motility phenotype is important in certain pathogenic but not commensal *E. coli* strains and that this is mediated by increased physical loads on the flagellar motor. During infection, flagella act as an antigen that the immune system can quickly recognise and mount a response to [112]. Laganenka et al. [108] therefore also hypothesize that flagellar mechanosensing aids in successful host colonisation by increasing expression of flagella in the early stages of infection when motility is useful in promoting contact with the epithelial cells. The authors expect decreasing flagella expression in later infection stages, possibly via quorum sensing, to reduce the hosts immune response to the flagella [108]. Mechanosensors such as flagella and their associated signalling systems allow for careful regulation of virulence, incorporating information from both chemical and physical cues. This allows pathogenic *E. coli* such as EHEC to produce the appropriate response to a highly dynamic and hostile host environment, promoting their survival and persistence.

Vibrio cholerae

The Vibrionaceae represent a diverse family of marine bacteria that can exist in a free-living planktonic state in the water column and also in a surface-attached state in shellfish, copepods and phytoplankton (natural hosts) or indeed within human hosts where they are opportunistic pathogens [113]. As such, the sensing of surface attachment is of critical importance in determining appropriate changes in gene expression. In planktonic *Vibrio* cells, swimmer cell morphology predominates, with motility being driven by a single polar flagellum that is powered by the sodium motive force (SMF) [114]–[116].

Inhibition of flagellar rotation through surface contact is thought to disrupt ion flux through the flagellar motor, leading to a transient membrane hyperpolarisation [117]. In *Vibrio cholerae*, deletion of the flagellar motor and chemical disruption of the SMF prevent this hyperpolarisation, thereby blocking the ability of *V. cholerae* to form robust monolayers [117]. Importantly, it has been shown

346 that *V. cholerae* mutants with defective motility display enhanced expression of ToxT-regulated
347 virulence factors including cholera toxin, cell-associated haemolysin and the toxin co-regulated pilus
348 [118]. Chemical inhibition and deletion of the NADH ubiquinone oxidoreductase Nqr, which functions
349 as a sodium extruding pump, were shown to hyperactivate *V. cholerae* ToxT [119]. Surface-attached
350 *V. cholerae* produces biofilms encased in exopolysaccharide (EPS) matrix, the production of which is
351 facilitated by two *vps* (*Vibrio* polysaccharide) gene clusters [120]. *V. cholerae* mutants lacking the
352 major flagellin FlaA display constitutive production of EPS, through loss of the ability to sense surface
353 contact, a phenotype which is mediated through phosphorylation of the biofilm master regulator VpsR
354 [121]. Deletion of motor components MotB and MotY in the Δ*flaA* background restored wild type
355 levels of EPS production indicating that both a functional flagellum and the motor-produced SMF are
356 required for fine control of EPS production. The authors observed that the non-flagellated, EPS
357 overproducing Δ*flaA* mutant produced reduced amounts of cholera toxin and toxin co-regulated pilus
358 and was defective for colonisation in a mouse model, highlighting the importance of surface sensing
359 for optimal virulence [121]. Regulation of the switch from swim to stick, with the aforementioned
360 activation of virulence, is also subject to control via the nucleotide second messengers c-di-GMP and
361 guanosine pentaphosphate ((p)ppGpp). C-di-GMP functions by synergistically activating VpsT an
362 upstream activator of VpsR [122] and also manipulating the conformation of the VpsR/σ70-RNAP
363 complex at bound promoters [123]. All three (p)ppGpp synthases possessed by *V. cholerae*, RelA, SpoT
364 and RelV were required for *vpsR* transcription, while only RelV was required for transcription of *vpsT*
365 demonstrating that control of biofilm/attachment and virulence genes is also responsive to nutritional
366 status through stringent response control [124].

367

368 ***Vibrio parahaemolyticus***

369 In the closely related pathogen *Vibrio parahaemolyticus*, swimmer cells behave similarly to those of *V.*
370 *cholerae* with a single SMF driven polar flagellum propelling the bacterium through liquid media [115].

Growth on solid surfaces however induces a marked change in cellular physiology with the formation of filamentous, peritrichously flagellated cells [114]. Physical, chemical and genetic disruptions in polar flagellum driven motility lead to activation of the peritrichous lateral flagella, encoded by the *laf* genes [125]. In contrast to the polar flagellum, *laf* flagella are driven by the proton motive force [115].

Second messenger driven signalling has also been implicated in regulating the switch from planktonic to attached lifestyles in *V. parahaemolyticus*. For example, ScrC possesses both diguanylate cyclase and phosphodiesterase activity, with phosphodiesterase activity predominating in surface attached cells [126]. Two proximally encoded genes (*scrAB*) were found to be capable of modulating the function of ScrC, enabling reduction in the intracellular pool of c-di-GMP and facilitating the switch between swarming motility (*laf* expression) and biofilm associated EPS production via the *cpsA-J* gene cluster [126]. A screen for ScrABC regulated genes identified that in addition to the *laf* and previously identified *cps* genes, a regulator encoding gene *cpsQ* was affected [127]. It was found that *cpsQ* activated *cpsA-J* in a c-di-GMP-dependent manner. Lateral flagella and virulence factors including T3SS1 and the *N*-acetyl glucosamine binding protein GbpA were found to be oppositely regulated to *cpsA-J* indicating activation by ScrABC [127].

Concluding remarks

The investigation of mechanosensing is an exciting and developing field of inquiry that has great potential to improve our understanding of pathogenic bacterial behaviour within the host. In this review, we have discussed the main mechanisms bacteria use to sense mechanical stimuli. These include flagellar impedance, resistance to type IV pili retraction and specific surface-bound adhesins. These sensory mechanisms are known to feed into a variety of signal transduction pathways, often acting by varying the levels of intracellular second messengers such as cAMP in *P. aeruginosa* and c-di-GMP in *P. aeruginosa* and *Vibrio* species. Signals carried through these and other mechanosensing linked pathways act on a wide range of different virulence-associated genes. These include genes encoding additional attachment factors, toxins, secretory systems and motility organelles. These allow

the bacteria to spread rapidly across surfaces, invade host cells, and inject effector molecules and release toxins which improve the success of pathogenic bacteria.

The prospect of future study in this field is exciting and there are many areas we look forward to seeing developed further. These include a more detailed understanding of the conformational changes in flagellar components as they are exposed to physical loads and how these allow for signal generation, a more complete understanding of how mechanosensing is incorporated with other sensory information such as sensing of host molecules and nutrients to fit into the wider picture of bacterial virulence modulation, further exploration of other adhesins which may play similar roles to PilY1 as mechanosensors and an expansion of the range of organisms whose mechanosensory systems are studied to learn more about how well-conserved mechanisms are across different bacterial strains and species.

Accurate sensing of environmental conditions is vital to allowing both pathogenic and non-pathogenic bacteria to produce an appropriate behavioural response to their surroundings. Not only is this important to bacterial survival but improved scientific understanding of this area could lead to new treatments and therapies. A more complete picture of how mechanosensory systems in pathogens activate virulence gene expression could allow for the development of new anti-virulence interventions which target these systems, disrupting pathogenesis at the outset and improving patient outcomes.

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Figure 1: Contact dependent sensing in *Pseudomonas aeruginosa.* 1a) Contact with, and binding to, a surface triggers the halting of flagellar rotation via interaction between components of the flagellar machinery and FimV. 1b) Contact with a surface allows type IV pili to attach, causing resistance to rhythmic pili retraction. This activates the Chp chemosensory system, triggering an interaction with FimV. 2) FimV causes an increase in production of cAMP, increasing its intracellular concentration. 3) cAMP binds to the Vfr virulence regulator which regulates the activity of over 200 virulence genes. 4a) Genes positively regulated by Vfr include several proteases, toxins and surface localised virulence factors such as the type II and III secretion systems. 4b) The type IV pilus itself is also positively regulated by Vfr and so gives rise to a positive feedback loop of increased surface detection signal and virulence expression. 5) Vfr promotes expression of FimS-AlgR system. 6) AlgR upregulates expression of PilY1, which in turn feeds back into FimS-AlgR, PilJ and Vfr to autoregulate its expression. 7a) PilY1 signals through SadC to increase c-di-GMP production. 7b) Surface growth signals are recognised by WspA and transmitted via the other components of the Wsp system (WspBCDEF) resulting in phosphorylation of WspR. Phosphorylated WspR then synthesises c-di-GMP. 8) C-di-GMP activates biofilm formation, suppresses cell motility and triggers virulence activation.

Figure 2: Contact dependent sensing in *Proteus mirabilis*. 1) Impedance of flagellar rotation signals surface contact. 2) This signal triggers the expression of 50 genes, resulting in the differentiation of small swimmer cells into elongated swarmer cells. These swarmer cells have increased virulence and numbers of flagella, improving their survival on a host surface. 3) ZapA is upregulated, producing a metalloprotease that is able to degrade antibacterial peptides utilised by the immune system of the host organism. 4) HpmB, the first gene in the HpmBA operon, is upregulated. This triggers the production of haemolysin, a major virulence factor responsible for aiding cell invasion and tissue damage of host cells.

Figure 3: Contact dependent sensing in *Escherichia coli.* 1) Shear forces result in GrlA transitioning from the inner cell membrane to the cytoplasm. 2) GrlA activates expression of Ler, which in turn activates expression of the rest of the LEE operon. 3) Expression of the LEE genes results in the assembly of the type III secretion system. This virulence factor allows the injection of specific effector molecules into a host cell, altering the host cytoskeleton and mediating disease. Remodelling of the host allows for close attachment of EHEC, improving survival.

Figure 4: Contact dependent sensing in *Vibrio cholerae.* 1a) Surface contact inhibits the rotation of the flagella, causing disruption to the flow of ions through the flagellar motor. This results in transient hyperpolarisation of the membrane. 1b) C-di-GMP also acts to control virulence gene regulation. C-di-GMP activates VpsT which in turn activates VpsR which binds with RNA polymerase to transcribe target genes. 1c) ((p)ppGpp) acts to control virulence regulation by regulating the expression of the aforementioned VpsT and VpsR proteins. 2) Genes are upregulated in response to these multiple sensory inputs. These include virulence genes encoding factors such as cholera toxin, haemolysin and attachment factors such as the toxin co-regulated pilus. 3) Motility associated genes such as those that encode flagellar components are downregulated by this signalling.

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