PLOS | NEGLECTED TROPICAL DISEASES

REVIEW

Vibrio cholerae Biofilms and Cholera Pathogenesis

Anisia J. Silva*, Jorge A. Benitez*

Morehouse School of Medicine, Department of Microbiology, Biochemistry and Immunology, Atlanta, Georgia, United States of America

* asilva-benitez@msm.edu (AJS); jbenitez@msm.edu (JAB)

Abstract

Vibrio cholerae can switch between motile and biofilm lifestyles. The last decades have been marked by a remarkable increase in our knowledge of the structure, regulation, and function of biofilms formed under laboratory conditions. Evidence has grown suggesting that *V. cholerae* can form biofilm-like aggregates during infection that could play a critical role in pathogenesis and disease transmission. However, the structure and regulation of biofilms formed during infection, as well as their role in intestinal colonization and virulence, remains poorly understood. Here, we review (i) the evidence for biofilm formation during infection, (ii) the coordinate regulation of biofilm and virulence gene expression, and (iii) the host signals that favor *V. cholerae* transitions between alternative lifestyles during intestinal colonization, and (iv) we discuss a model for the role of *V. cholerae* biofilms in pathogenicity.

Citation: Silva AJ, Benitez JA (2016) *Vibrio cholerae* Biofilms and Cholera Pathogenesis. PLoS Negl Trop Dis 10(2): e0004330. doi:10.1371/journal.pntd.0004330

Editor: Stephen Baker, Oxford University Clinical Research Unit, VIETNAM

Published: February 4, 2016

Funding: Research in the authors' laboratory is funded by Public Health Service Grants AI103693 and AI104993 from the National Institute of Allergy and Infectious Diseases (http://www.niaid.nih.gov). The funders had no role in study design, data collection and analysis, decision to publish, or preparation of the manuscript.

Competing Interests: The authors have declared that no competing interests exist.

Introduction

The water-borne diarrheal disease cholera is caused by the gram-negative and motile bacterium *Vibrio cholerae* of serogroup O1 and O139. *V. cholerae*, as other members of the *Vibrionaceae* family, are common inhabitants of aquatic ecosystems. In regions where cholera is endemic, occurrence of the disease follows a seasonal pattern that correlates with climatic changes [1–8]. Import of *V. cholerae* O1 into nonendemic areas with poor sanitation commonly results in rapid dissemination of the disease through a fast fecal–oral route that takes advantage of the transient hyperinfective stage of *V. cholerae* present in fresh cholera stool [9–12]. *V. cholerae* O1 can be divided into two biotypes, classical and El Tor, which differ in the severity of clinical symptoms and the expression and regulation of major virulence factors [13]. Humans have experienced seven cholera pandemics. The seventh and current pandemic is characterized by the predominance of the O1 serogroup of the El Tor biotype, with periodic emergence of serogroup O139, which originated from the El Tor biotype and exhibits a new lipopolysaccharide (LPS) and a capsule [14].

Virulence Factors

The two major virulence factors expressed by *V. cholerae* O1 and O139 are (i) cholera toxin (CT), an AB_5 family ADP-ribosyltransferase responsible for the profuse rice-watery diarrhea typical of this disease [13], and (ii) the toxin-coregulated pilus (TCP), a type IV pilus that

mediates adherence and microcolony formation and is required for intestinal colonization in neonate mice and humans [15–17]. The genes encoding the CT subunits *ctxA* and *ctxB* constitute an operon within the prophage form of the filamentous phage CTXΦ [18]. The genes required for TCP biogenesis form a large cluster known as the *V*. *cholerae* pathogenicity island (VPI) or TCP island [19]. Within this cluster, *tcpA* encodes the major pilus subunit.

Also important for the pathogenicity of the cholera bacterium is the expression of a sheathed polar flagellum driven by sodium motive force (SMF) [20]. Flagellar motility is a complex phenotype that requires (i) the synthesis, export, and assembly of the flagellum and its motor; (ii) conversion of SMF to flagellum rotation work; and (iii) control of the direction of flagellum rotation by chemotaxis. The expression of motility requires a hierarchical regulatory cascade that involves the alternative RNA polymerase subunits σ^{54} and σ^{28} and the σ^{54}-dependent transcriptional activators FlrA and FlrC [21]. In addition, evidence has grown suggesting that flagellar motility participates in the regulation of virulence gene expression. For instance, mutations or chemical inhibitors that result in a paralyzed flagellum enhance the transcription of *ctxA* and *tcpA* [22–26]. The mechanism by which cessation of motility enhances virulence gene expression is unknown.

Stress Response

V. *cholerae* has evolved to effectively colonize disparate ecological niches: the nutrient-rich human small intestine and aquatic environments. In the aquatic environment, *Vibrios* must withstand diverse physical, chemical, and biological stresses that include nutrient limitation, extreme temperatures, oxidative stress, bacteriophage predation, and protozoan grazing [27,28]. In the gastrointestinal tract, *Vibrios* are exposed to low pH, bile acids, elevated osmolarity, iron limitation, antimicrobial peptides, and intermittent nutrient deprivation [29]. Thus, both environments pose common and specific challenges to bacterial growth and multiplication. The human small intestine, nevertheless, provides a superior bounty of nutrients compared to aquatic environments. Consistently, *V*. *cholerae* can grow to high titers in the human gut, and cholera patients can shed 10^7–10^9 virulent *Vibrios* per mL in the rice-watery stool [12]. In order to reach high titers in the gut, *V*. *cholerae* must overcome as many stressful conditions as it requires to survive and persist outside the human host. Proof of this is that disruption of genes encoding the general stress response regulator RpoS (σ^S) or the RNA polymerase σ^E subunit (RpoE) that mediates the envelope stress response results in significant attenuation of *V*. *cholerae* virulence and its capacity to colonize the small intestine [30,31]. Thus, whether in the human host or in the aquatic environment, the cholera bacterium employs common survival strategies. These stratagems involve (i) the activation of general and specific stress responses, (ii) expression of flagellar motility and chemotaxis, (iii) attachment to surfaces, (iv) development of multicellular sessile communities, and (v) detachment. Particularly critical to *V*. *cholerae* survival in the host and estuarine waters is its ability to switch between motile (planktonic) and sessile (biofilm) lifestyles in response to chemical and physical changes in the extracellular milieu.

V. cholerae Biofilms

Biofilms are microbially derived sessile communities characterized by cells that are attached to a substratum, an interface, or to each other; are embedded in a self-produced matrix; and exhibit an altered phenotype with respect to growth rate and transcription profile [32,33]. This definition includes communities of *Vibrios* anchored to abiotic surfaces or to biotic substrata such as the human intestinal mucosa or the chitinous exoskeleton of crustaceans, *Vibrio* aggregates in suspension, floccules, and pellicles formed at the liquid–air interface of static cultures.

It has been established that *V*. *cholerae* cells in planktonic, monolayer, and mature biofilm stages differ in their global transcription profile [34,35]. A major event in the transition from planktonic to biofilm lifestyle is the down-regulation of motility gene expression and induction of genes required for the biosynthesis of the biofilm extracellular matrix [34,35]. We note that the signaling pathway by which an initial attachment to a surface induces significant changes in the transcriptome is unknown. In the mature biofilm microenvironment, cells are packed within a smaller volume, and nutrient accessibility and the elimination of toxic metabolic products is limited by diffusion. These conditions favor an early entry of cells into quorum sensing mode and stationary phase. As an example, the cholera autoinducer 1 was shown to accumulate to a higher concentration in biofilms compared to planktonic cells, resulting in earlier expression of the quorum sensing regulator HapR [36]. In turn, HapR enhances the expression of the stationary phase sigma factor RpoS [37]. Thus, the mature biofilm exhibits a gene expression pattern that favors resistance to environmental stressors.

The regulation and structure of biofilms formed under laboratory conditions has been the subject of much study and several reviews [38–40]. It is well established that the intracellular concentration of the second messenger cyclic diguanylic acid (c-di-GMP) controls the transition between *V*. *cholerae* planktonic and biofilm lifestyles [41–46]. c-di-GMP is synthesized from GTP by the activity of diguanylate cyclase (DGC) exhibiting GGDEF domains and degraded to GMP by phosphodiesterases (PDE) exhibiting EAL or HD-GYP domains [47]. The *V*. *cholerae* genome encodes 31 GGDEF, 22 EAL, 9 HD-GYP, and 10 combined GGDE-F-EAL domain proteins [48]. Most of these proteins display a modular architecture with added sensor, effector, and DNA binding domains.

Three major regulators sense the intracellular concentration of c-di-GMP: the σ^{54}-dependent activator FlrA required for the expression of flagellar motility [21,49] and the biofilm activators VpsR [50] and VpsT [51,52]. Five membrane-bound DGC (CdgA, H, L, K, and M) act additively to increase the c-di-GMP pool and promote dimerization and activation of VpsT to induce biofilm formation [46]. The *V*. *cholerae* genome also encodes five proteins containing PilZ domains, a separate family of c-di-GMP binding proteins [53]. The role of PilZ domain proteins in regulating motility, biofilm, and virulence is unclear. Deletion of three genes encoding PilZ domain proteins resulted in reduced motility, diminished biofilm formation, and intestinal colonization [53]. The negative effect of these deletions on motility and biofilm formation is unexpected, given that these cellular processes are inversely regulated by c-di-GMP. It is possible that the deleted PilZ proteins affect motility and colonization by a mechanism unrelated to c-di-GMP.

The genes (*vps*) responsible for making the *V*. *cholerae* exopolysaccharide matrix are located in two clusters (*vpsU*, *vpsA-K*) and *vpsL-Q* on *V*. *cholerae* chromosome I [54]. These clusters comprise two operons in which *vpsA* and *vpsL* are the first genes of operon I and II, respectively [54]. A third gene cluster, *rbmA-F*, located between the *vpsA-K* and *vpsL-Q* operons and *bap*1 encode protein components of the biofilm matrix [55]. RbmA is only expressed on the surface of cells that make the exopolysaccharide and functions to enhance cell-to-cell adhesion [56]. In addition, RbmA can undergo limited proteolysis to a form capable of interacting with cells not expressing *vps*, thereby recruiting planktonic cells to the growing biofilm [57]. Bap1 promotes adherence of the developing biofilm to a surface [56,58,59], and RbmC cooperates with Bap1 in the formation of flexible envelopes that grow as cells divide and stabilize the biofilm [56]. Deletion of *rbmA*, *rbmC*, and *bap*1 results in diminished biofilm formation in vitro [55].

The expression of genes in the *vps* and *rbm* clusters is under positive transcription regulation by VpsR and VpsT [40]. When the intracellular concentration of c-di-GMP is high, allosteric activation of VpsR and VpsT enhances the expression of genes required to make the

biofilm matrix [60]. In parallel, c-di-GMP binds to FlrA to inhibit its activity and diminish flagellar gene expression [49].

In addition to exopolysaccharide and proteins, the biofilm matrix contains extracellular DNA (eDNA). The eDNA has been suggested to contribute to the structural stability of biofilms, though its precise interaction with other components of the matrix is unclear [61]. In *V. cholerae*, the level of eDNA is regulated by nucleases *dns* and *xds*, the latter gene a member of the Pho regulon [62]. A double mutant lacking both nucleases produced enhanced biofilm as a consequence of reduced detachment [63]. It has also been suggested that eDNA can serve as a source of phosphate to biofilm cells [64]. Thus, phosphate starvation could function as a signal for eDNA degradation and biofilm dispersion. This interpretation is consistent with results showing that phosphate limitation negatively affects biofilm formation [65,66]. We note, however, that degradation of eDNA could potentially increase the concentration of cytidine in the biofilm, an allosteric inhibitor of the LacI-family regulator CytR reported to repress *vps* expression and biofilm formation [67]. Hence, much remains to be learned on the role of eDNA, phosphate regulation, and nucleoside catabolism in biofilm formation and dispersal.

Evidence for Biofilm Formation during Infection

Microscopic observation of in vivo-formed biofilm-like aggregates

Early microscopic examination of *V. cholerae* in association with the intestinal mucosa of adult and infant rabbits revealed patches of *Vibrios* adherent to the mucous coat and along the villi [68–71]. More recently, the use of confocal and intravital two-photon microscopy confirmed the localization of infecting *Vibrios* in the form of clonal microcolonies attached along the villous axis and crypts [72]. Further, examination of human fresh cholera stool reveals the presence of *V. cholerae* in the form of both planktonic cells and biofilm-like structures consisting of large clumps of cells [73,74]. However, the composition and architecture of *V. cholerae* microcolonies and biofilm-like aggregates formed in vivo has not been established.

Common genetic determinants of biofilm development and intestinal colonization

There is plentiful evidence suggesting that the capacity of *V. cholerae* to develop biofilms is critical to intestinal colonization. The genes required for the adoption of both *V. cholerae* lifestyles are expressed during infection in the rabbit ileal loop model [75]. In fact, some biofilm genes (i.e., *vpsA*, *rbmA*) were expressed at a higher level in vivo compared to LB medium [75]. In a suckling mouse single strain infection colonization assay, planktonic *vps* mutants of *V. cholerae* O1 (El Tor), which are defective for biofilm formation in vitro, exhibit diminished recovery from intestinal tissue compared to wild type [76]. Deletion of *rbmA* significantly diminished intestinal colonization, while inactivation of *rbmC* and/or *bap*1 had no effect [76]. This finding suggests that biofilms formed during infection may consist primarily of cell aggregates that do not progress beyond the RbmA-dependent clustering stage or that other factors can functionally replace Bap1 and RbmC for biofilms formed in vivo. The above data contradicted an earlier competitive colonization study suggesting that *vps* expression is not required for intestinal colonization by *V. cholerae* O139 [77]. Possible explanations for the conflicting results are (i) an unrecognized role of the O139 LPS in adherence to the intestinal mucosa or (ii) masking of the O139 *vps* defect by *Vibrio* exopolysaccharide (Vps) produced by the wild type co-inoculated strain. Nevertheless, expression of *vps* genes were required for *V. cholerae* O139 intestinal colonization in the fruit fly *Drosophila melanogaster*, a novel oral infection model that exhibits cholera toxin-dependent lethality and mimics human infection [78,79].

Inhibition of motility, which diminishes biofilm formation in vitro, also negatively affects colonization of the small intestine [24,77,80]. Nonmotile mutants exhibit reduced attachment and monolayer formation on abiotic surfaces as well as adherence to the intestinal epitlelium in animal models [24,81–83]. The mechanism by which flagellar motility affects surface attachment is not fully understood. Initial studies suggested that adherence could be modulated by sodium flux through the flagellar motor and membrane potential [80,84]. A later study suggested that the flagellum allows *Vibrios* to swim along a surface and act synergistically with pili in a scanning mode for strong surface–pili interactions [85]. The above mechanisms are difficult to dissect, as conditions that slow the flagellum (i.e., tethering the cell to a surface, high viscosity) could diminish sodium flux through the motor and perturb the membrane potential. Likely, both mechanisms can contribute to bacterial adherence. The mannose-sensitive hemaglutinin (MSHA) is important for biofilm development on borosilicate surfaces [81,86] and on the exoskeletons of the planktonic crustacean *Daphnia pulex* [87]. In vitro, the polar flagellum was proposed to act synergistically with the MSHA to promote surface skimming and attachment [85]. However, deletion of *mshA* does not affect intestinal colonization [16,17]. Thus, the flagellum could act in concert with other pili to promote attachment during infection. We suggest that the TCP pilus, which mediates microcolony formation and attachment to polarized Caco-2 cells [88–91], could act synergistically with the flagellum to promote adherence to intestinal cells.

Altogether, the fact that production of Vps, RbmA, and motility are required for efficient colonization of the small intestine suggests that *V*. *cholerae* must be capable of adopting both motile and biofilm lifestyles during the infective process to successfully colonize the small bowel.

Changes in the *V*. *cholerae* c-di-GMP pool during infection

Fluctuations in the intracellular c-di-GMP pool during infection suggests that *V*. *cholerae* senses environmental cues in the small intestine that favor lifestyle transitions from sessile to motile and vice versa. It was reported that an increase in the c-di-GMP pool represses virulence gene expression [92–94]. These finding led to a model in which *V*. *cholerae* enters the small intestine in a stage characterized by an elevated c-di-GMP pool such as a biofilm, but induction of PDE and/or repression of DGC activities act to lower the c-di-GMP pool to achieve maximal expression of motility and virulence genes [48]. However, recombination-based in vivo expression technology (RIVET) identified genes specifically expressed late in infection encoding GGDEF domain proteins with DGC activity [95]. The authors suggested that *V*. *cholerae* can increase its c-di-GMP pool late in infection, a condition that promotes biofilm development, prior to exiting the host in preparation for life in the aquatic environment. This model does not explain, however, why planktonic *vps* mutants exhibit diminished capacity to establish infection in the suckling mouse model. Recent studies suggest *V*. *cholerae* may occupy distinct niches along the small intestine, requiring location-specific factors [72,96]. We suggest that *Vibrios* must respond to changes in the chemical composition of their surroundings during infection by switching between sessile and motile lifestyles. This ability could provide fitness and explain why both planktonic nonmotile and *vps* mutants exhibit diminished intestinal colonization capacity.

The Coordinate Regulation of Virulence Gene Expression and Biofilm Development

The regulation of virulence gene expression has been the subject of extensive research and recent reviews [97,98]. Expression of CT and TCP is regulated by a complex regulatory

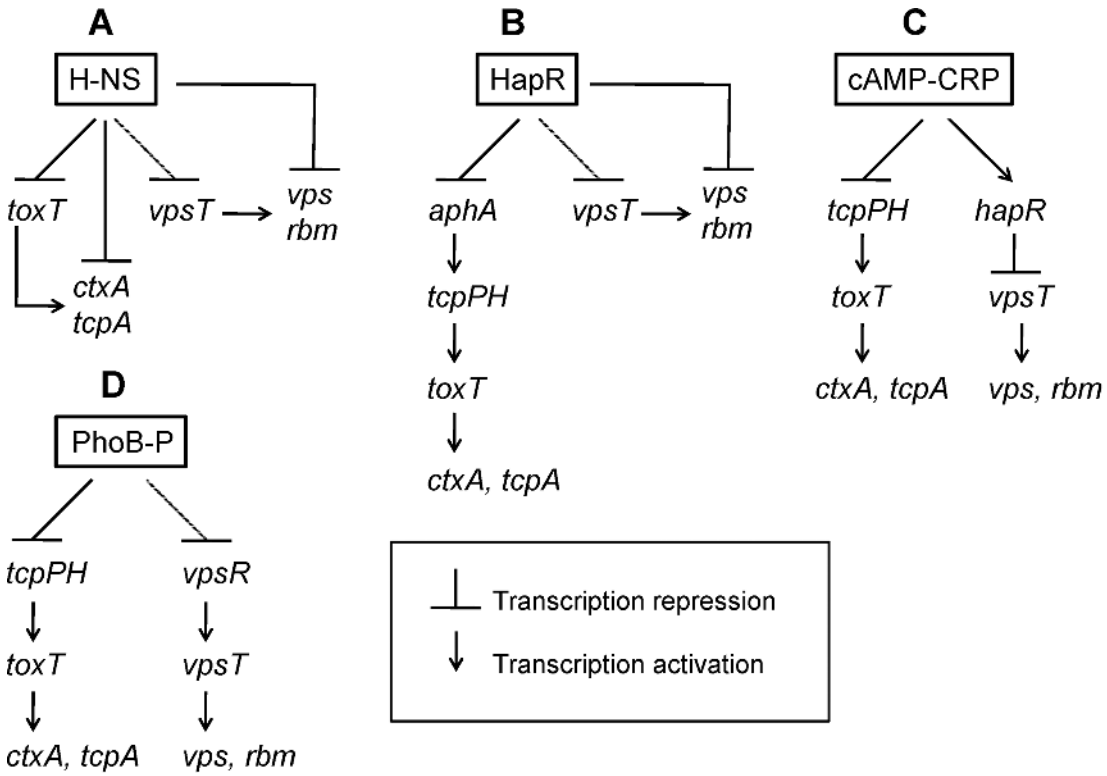

Fig 1. Coordinate regulation of virulence gene expression and biofilm development. (A) The nucleoid organizer H-NS silences the transcription of *ctxA* and *tcpA* directly and via repression of *toxT*. Expression of *ctxA* and *tcpA* is made possible by the action of proteins ToxT and IHF that function as antirepressors. H-NS silences the transcription of *vpsT* and downstream *vps* and *rbm* genes. Activation of biofilm genes is made possible by a VpsR- and VpsT-dependent antirepression cascade. (B) At high cell density, the quorum sensing regulator HapR diminishes *ctxA* and *tcpA* expression by repressing *aphA*. HapR terminates the transcription of *vpsT* to repress biofilm formation. (C) Depending on carbon source type and availability, the cAMP receptor protein (CRP) negatively regulates virulence and biofilm formation by repressing the transcription of *tcpPH* and activating HapR. D. Phosphate limitation triggers phosphorylation of PhoB which diminishes virulence gene expression and biofilm by repressing *tcpPH and vpsR*, respectively.

doi:10.1371/journal.pntd.0004330.g001

network (Fig 1). At the top of the Tox regulatory cascade, regulators AphA and AphB enhance the transcription of the transmembrane regulators TcpP and TcpH [99,100]. TcpP/H, in concert with transmembrane regulators ToxR/S [101,102], activate the expression of the soluble AraC-family regulator ToxT [103]. Finally, ToxT interacts with the *ctxA* and *tcpA* promoters to activate the production of CT and TCP [103]. The dependence of *ctxAB* and *tcpA* expression on the upstream regulators ToxR/S, TcpP/H, and ToxT was confirmed in vivo using the suckling mouse model and RIVET [104].

Numerous signal transduction pathways simultaneously feed sensory information into the Tox cascade and control the c-di-GMP pool to coordinate the expression of major virulence factors and biofilm development (Fig 1). These regulatory connections point to the conclusion that signals that enhance virulence gene expression and biofilm formation are present in the environment of the small intestine creating the evolutionary pressure to coordinate these cellular processes.

Transcriptional silencing of virulence and biofilm formation by the histone-like nucleoid structuring protein (H-NS)

H-NS is a highly abundant protein that functions as a nucleoid organizer and a transcriptional silencer at promoters exhibiting AT-rich, highly curved DNA. It preferentially silences the

transcription of virulence factors acquired by horizontal gene transfer [105]. In *V*. *cholerae*, transcription of the xenogenic genes *ctxA* and *tcpA* is silenced by H-NS (Fig 1A) [106]. Repression of *ctxA* and *tcpA* is antagonized by ToxT and the integration host factor (IHF) [107–109]. The *vps* and *rbm* genes are also silenced by H-NS and are expressed or reset back to silent depending on environmentally induced fluctuations in the c-di-GMP pool (Fig 1A) [110–114]. We showed that activation of VpsR by c-di-GMP antagonizes H-NS repression at the *vpsT* promoter [112]. Then, expression and allosteric activation of VpsT by c-di-GMP antagonizes H-NS repression at the downstream *vps* and *rbm* promoters [112].

Chromatin immunoprecipitation and parallel DNA sequencing (ChIP-Seq) showed a significant trend for H-NS to cluster at regions of the chromatin involved in the expression of virulence (*ctxAB*, TCP island), surface attachment, and biofilm formation (*vps*, *rbm*) [112,113]. Recent studies have led to the view that H-NS organization of the chromatin and transcriptional silencing are interrelated functions in which gene regulation drives nucleoid organization [115]. Clustering of H-NS at sites of the chromatin encoding virulence factors and genes required to make the biofilm matrix could bring these regions into proximity rendering their coordinate regulation more effective. Further, H-NS clustering at these sites could function to synchronize virulence and biofilm formation in response to environmental conditions that affect DNA superhelical density, such as temperature, pH, osmotic shifts, transitions from aerobiosis to anaerobiosis, and starvation [116]. Thus, the above studies suggest that H-NS coordinates biofilm and virulence gene expression at both the transcription initiation and chromatin organization levels.

Quorum sensing

Quorum sensing is a cell–cell communication process in bacteria that involves the production, release, and subsequent sensing of signaling molecules termed autoinducers. In *V*. *cholerae*, two autoinducer/sensor systems have been identified. The major system consists of cholera autoinducer 1 (CA-1) and its cognate receptor, CqsS, while a second system consists of autoinducer 2 (AI-2) and its cognate receptor, LuxPQ [117,118]. At low cell density, multiple, redundant small regulatory RNAs (sRNAs or *qrr*) enhance expression of the regulator AphA and destabilize the mRNA encoding the regulator HapR [118,119]. Accumulation of CAI-1 at high cell density results in termination of sRNA transcription, down-regulation of *aphA*, and expression of *hapR* [118,119]. The master quorum sensing regulator HapR lowers virulence gene expression by inhibiting the transcription of *aphA* (Fig 1B) [120]. Quorum sensing negatively controls biofilm formation through the regulators AphA and HapR [118]. At low cell density, AphA enhances the expression of the biofilm activator VpsT [121]. At high cell density, HapR diminishes biofilm formation by lowering the intracellular c-di-GMP pool and repressing *vpsT* (Fig 1B) [122]. Thus, in contrast to other bacterial pathogens, quorum sensing acts in *V*. *cholerae* to repress biofilm formation and virulence gene expression.

Carbon source and nutrient limitation

Bacteria respond to the availability of sugars in the medium through a phosphoryl transfer cascade known as the phosphoenolpyruvate (PEP) phosphotransferase system (PTS) [123]. In the PTS, sugar transport and phosphorylation occur at the expense of PEP through a phosphoryl cascade involving the pathway-specific proteins enzyme I (EI) and HPr, and sugar-specific enzyme II (EII) complexes [123]. The different EII complexes are characterized by their domains (A, B, C), present either on a single or distinct polypeptide chains. In the *Enterobacteriaceae*, phosphorylated glucose-specific EIIA activates adenylate cyclase to make cAMP, which binds to the cAMP receptor protein (CRP) to induce or repress gene expression [123].

In *V*. *cholerae*, the expression of virulence genes is negatively modulated by CRP, which acts to repress transcription of *tcpPH* [100] and activate the expression of HapR (Fig 1C) [124–126]. A mutant lacking EI of the PTS showed diminished colonization in neonate and germ-free adult mice models, suggesting that individual components of the PTS can exert additional regulation over virulence gene expression [127,128]. Carbon source modulates the expression of VpsR and VpsT by controlling the expression of HapR via CRP [124,126,129] and the levels of phosphorylated intermediates of the PTS where phospho-EI and phospho-HPr act to repress *vps* expression [128,130,131].

Phosphate limitation

Freshwater and estuarine ecosystems where *V*. *cholerae* can survive and persist outside the human host are limited in phosphate content. Similarly, phosphate is a limiting nutrient in the small intestine [62]. Bacteria respond to phosphate limitation through the PhoR/PhoB two-component regulatory system [132]. Under conditions of phosphate limitation, the histidine kinase PhoR interacts with the phosphate transport system (Pst) to activate PhoB by phosphorylation. Phosphorylated PhoB then modulates the transcription of a set of genes known as the Pho regulon [132]. During infection, phosphorylated PhoB diminishes *ctxA* and *tcpA* expression by binding to the *tcpPH* promoter to repress transcription initiation [133]. Phosphate limitation and PhoB negatively control biofilm formation by lowering the expression of VpsR and modifying the c-di-GMP pool [65,66].

Host Signals That Coordinate Virulence Gene Expression with Biofilm Development

Numerous physical and chemical cues in the gut (i.e., temperature, pH, oxygen tension, osmolarity, bile salts, antimicrobial peptides) can impact the infective process. It is likely that all these factors, at least indirectly, influence virulence and biofilm formation. Compounds that perturb the cell envelope can generate additional stresses, resulting in elevated expression of RNA polymerase subunits σ^E and σ^S [134]. Intestinal bile exhibits these properties and has received abundant consideration as a host-specific signal that can potentially modulate *Vibrio* behavior in the gut. Intestinal bile is a complex mixture of bile acids, cholesterol, and unsaturated fatty acids and is subject to numerous chemical transformations in the gastrointestinal tract (i.e., removal of amino acid side chains, oxidation, hydroxylation, and dehydroxylation). Crude bile or sodium cholate was found to enhance biofilm formation in a VpsR-dependent manner [135]. This observation is consistent with the recent finding that a mixture of bile acids increased the intracellular c-di-GMP pool, an effect that was quenched in the presence of bicarbonate [136]. Surprisingly, the individual bile salt taurocholate was found to promote biofilm dispersal rather than formation [137]. These differences may reflect the limited capacity of commercial bile preparations to represent the properties of bile secreted into the intestinal lumen.

Bile also modulates virulence gene expression. Treatment of *V*. *cholerae* with a crude ox bile extract resulted in diminished expression of CT and TCP [138]. Subsequent studies with purified bile components showed that unsaturated fatty acids repress the transcription of *ctxA* and *tcpA* [139]. Unsaturated fatty acids were shown to inhibit ToxT activity [140] and its binding to the *ctxA* and *tcpA* promoters [141]. Contrary to the effect of unsaturated fatty acids, bicarbonate enhanced the activity of ToxT and its binding to its target promoters [142,143]. Bile concentration is elevated in the lumen and low in the vicinity of the villi, while bicarbonate exhibits the opposite gradient. Thus, these molecules could act as a location-specific switch, modulating *V*. *cholerae* behavior during infection. In summary, the above studies suggest an

inverse regulatory model in which components of bile present in the intestinal lumen favor biofilm formation by enhancing the c-di-GMP pool and, in parallel, suppress the premature expression of the major virulence factors CT and TCP.

Colonization of the Small Intestine

The small intestine commences at the pyloroduodenal junction and ends at the ileocaecal junction and comprises, successively, the duodenum, jejunum, and ileum. The mucosal side of the small intestine is composed of absorptive polarized epithelial cells (enterocytes) organized in the form of finger-like projections or villi and mucin-secreting goblet cells covered by a protective mucus barrier. The protective mucus coat consists of a firmly adherent inner layer overlaying the villi and a loosely attached outer layer [144]. The thickness and biophysical properties of the mucus barrier varies along the gastrointestinal tract and is determined by the balance between its secretion rate and its erosion through enzymatic degradation and mechanical shear. The total mucus layer thickness is estimated to be 170–123 μM in the duodenum and jejunum and 480 μM in the ileum [145].

V. *cholerae* colonization of the small intestine has been extensively studied using the suckling mouse competitive colonization assay [146], infant rabbits [68,147], and rabbit ileal loops [68]. The use of the above animal models in combination with transposon or signature-tag mutagenesis and RNA-Seq has led to the discovery of multiple colonization factors [148–151]. Further, the suckling mouse colonization assay in combination with RIVET has identified genes that are specifically induced during infection [152–155]. Genes that specifically influence intra-intestinal growth fall into two broad categories: those encoding factors that enhance intestinal colonization (i.e., motility) and those that are stringently required for colonization, such as *tcpA* [15,156]. Activities of TCP that promote intestinal colonization include adherence to intestinal cells [90], microcolony formation [88,89], and secretion of the secondary colonization factor TcpF [157].

Contracting cholera involves the oral ingestion of virulent *Vibrios* capable of expressing TCP and CT in the form of planktonic cells or biofilms. *V*. *cholerae* cells in a biofilm exhibit a lower infective dose and outcompete their planktonic counterparts in the suckling mouse colonization assay [158]. The biofilm advantage is transient and does not require the intact biofilm architecture [158]. *V*. *cholerae* biofilms have been reported to be more resistant to acid inactivation [159]. In addition, biofilm-derived cells could be more effective in competing for limiting nutrients in the small intestine, as suggested by their elevated expression of their phosphate uptake system compared to planktonic cells [160].

Wild type (chemotactic, motile) *V*. *cholerae* preferentially colonizes the middle to distal small intestine [96,161,162]. In contrast, motile but nonchemotactic mutants exhibiting counterclockwise flagellum rotation colonized the entire length of the small intestine, while nonmotile or nonchemotactic mutants showing clockwise flagellum rotation exhibited diminished colonization [24,154,161]. It has been suggested that ingestion of hyperinfective biofilms can represent a natural mode of contracting cholera during outbreaks and a fast track for disease dissemination [12]. However, the fact that both motility and chemotaxis positively influence *V*. *cholerae* colonization capacity indicates that *Vibrios* within an infective biofilm must detach and switch to the planktonic lifestyle to effectively colonize the gut. Consistent with this view, Δ*dns*Δ*xds* nuclease-defective mutants that showed diminished detachment of cells from biofilms in vitro also exhibited reduced intestinal colonization capacity, presumably due to inefficient dispersal of the biofilm upon entering the intestinal lumen [63].

Vibrios detached from an incoming biofilm must swim toward the intestinal mucosa and penetrate the protective mucus barrier. Flagellar motility could facilitate bacterial attachment

to the protective mucus layer in cooperation with the N-acetylglucosamine-binding protein and colonization factor GbpA reported to mediate bacterial adherence to intestinal mucin [163–166]. Flagellar motility can also contribute to the initial penetration of the mucus gel. This view is suppported by the observation that pretreatment of mice with the mucolytic agent N-acetyl-L-cysteine partially restored colonization capacity to nonmotile mutants [72]. Though in the standard suckling mouse model motility is required for overall intestinal colonization, more refined microscopy techniques showed that *V*. *cholerae* colonization of the proximal and distal small intestine exhibit distinct requirements for motility and chemotaxis [72]. It is intriguing that motility was required for *V*. *cholerae* to reach the crypts of the proximal small intestine, but not the distal region protected by a thicker mucus gel [72]. Thus, the mechanism by which *Vibrios* reach their niche outside the intestinal lumen could involve additional factors. This notion is supported by studies showing that the polar flagellum is a less effective locomotion organelle in a high viscocity medium [167] and tends to break in the viscous mucus gel [166]. Penetration of the mucus barrier could be as well facilitated by flagellum-independent locomotion and/or the activity of mucolytic enzymes. A flagellum-independent surface translocation on semisolid media has been reported in *V*. *cholerae*, which required the production of wild-type LPS [168]. In addition, expression of the the the Zn-dependent metalloprotease hemagglutinin (HA)/protease [169,170] enhanced the penetration of a mucin gel in vitro in a column assay [170]. A recent in vitro study suggested that intestinal mucin represses the expression of *vps* genes [171]. We suggest that this effect could prevent the counterproductive formation of sessile cell aggregates and/or biofilms within the protective mucus layer.

Following penetration of the mucus barrier, *Vibrios* locate along the villous axis and crypts in the form of microcolonies [72]. Microcolony formation is thought to be mediated by TCP [89], which is also an adherence factor [90] and promotes biofilm formation on chitin [172]. A recent study showed that microcolonies observed along the villous axis and the crypts are clonal [72]. This finding contrasts with the mechanism by which TCP promotes the formation of nonclonal aggregates in vitro through pilus–pilus interactions [89]. Thus, TCP could contribute to microcolony formation in vivo by a mechanism distinct from pilus–pilus interaction and/or cooperate with additional factors. We do not know if microcolonies are embedded in an exopolysaccharide matrix similar to the biofilms formed in vitro. Further, while *vps* mutants show diminished colonization [76], it has not been determined if microcolony formation is *vps*-dependent.

Vibrios along the villous axis and crypts express CT, which binds to its GM_1 receptor in the apical membrane of intestinal epithelial cells and is internalized by endocytosis. Based on the inverse relationship between motility and virulence gene expression [24,25], cessation of motility upon bacterial attachment to the villi is expected to enhance CT expression. Toxin delivery at this site in close proximity to its GM_1 receptor is stimulated by low bile and elevated bicarbonate levels. This spatiotemporal pattern of CT expression is consistent with previous studies indicating that transcription of *ctxAB* in vivo is preceded by the expression of TCP [104]. Late in infection, *Vibrios* down-regulate the expression of major virulence factors and detach to disseminate throughout the small intestine or return to the aquatic environment [173].

Detachment

In the context of this article, we consider detachment the process by which cells in a sessile stage detach and switch to the planktonic (motile) lifestyle. The mechanism by which cells detach could include degradation of the substratum to which they are attached or cleavage of a protein or adhesin that anchors monolayers or multilayers of cells to a surface. In fully developed biofilm communities, detachment could be triggered by nutrient deprivation,

accumulation of specific metabolites or toxic products, or as a consequence of external signals. The effective dispersion of motile *Vibrios* from a mature biofilm would require a certain degree of degradation of the biofilm matrix that holds cells together.

The soluble HA/protease was hypothesized to function as a "detachase" during infection based on the observation that *hapA* mutants remained attached for longer periods to Henle-407 cells [174], exhibited enhanced adherence to differentiated mucin-secreting HT29-18N2 cells [175], and elevated association with intestinal tissue compared to wild type [176]. HA/protease is a mucinase that is activated by quorum sensing and RpoS [125,177]. The "detachase activity" of HA/protease could partly result from its mucinase activity [178]. In addition, HA/protease was shown to degrade the GbpA adhesin required for attachment of *V*. *cholerae* to intestinal mucin [179]. A proteomic analysis showed that HA/protease is present in the matrix of biofilms formed in vitro [59]. HA/protease was recently shown to cleave RbmA, but this event increased biofilm formation rather than dispersal, as the RbmA cleavage product functioned to recruit planktonic cells to the growing biofilm [180].

Reversal of a cell population from sessile to motile lifestyle is favored by environmentally induced downshifts in the c-di-GMP pool. Expression of HapR at high cell density and RpoS in stationary phase diminishes the c-di-GMP pool [37,122]. Lowering of the c-di-GMP pool enhances motility by increasing the activity of FlrA and diminishing the export of exopolysaccharides that stall the flagellum [49]. We have shown that transcription of *rpoN* encoding RNA polymerase σ^{54} subunit and *flrA* is directly diminished by H-NS and that expression of RpoS in the stationary phase counteracts this negative effect to enhance motility [181]. An RpoS-dependent activation of motility termed the "mucosal escape" response was first identified using the rabbit ileal loop model [182]. We have further shown that VpsT negatively impacts the mucosal escape response by repressing the transcription of *rpoS* [183]. In Fig 2, we unify these observations into a model for detachment involving quorum sensing, VpsT, and RpoS.

Model for the Role of Biofilms in Intestinal Colonization and Pathogenesis

The genes required for the expression of flagellar motility and the biosynthesis of the biofilm exopolysaccharide and protein matrix (i.e., *vps*, *rbm*) are necessary for the efficient colonization of the small intestine. Therefore, the capacity of *V*. *cholerae* to adopt both lifestyles during infection could provide fitness in the environment of the gut. As shown in Table 1, flagellar motility could provide *Vibrios* with the advantage of mobility and capacity to spread along the gastrointestinal tract. On the other hand, biofilm formation could provide a mechanism of resistance to the host innate defense mechanism, facilitate a fast fecal–oral transmission route, and increase the fitness of those *Vibrios* that are directly shed back into the aquatic environment.

In Fig 3, we provide a schematic view of the intestinal colonization process that takes into consideration the potential role of biofilm intake, their formation during infection, and excretion to the environment. *Vibrios* that enter the host in the form of a biofilm (Fig 3A) have a competitive advantage compared to planktonic cells. Planktonic cells detached from an infecting biofilm initially interact with the protective mucus barrier and move toward the underlying epithelium (Fig 3B). *Vibrios* are prevented from forming biofilm-like aggregates within the mucus gel by repression of *vps* genes (Fig 3B) [171]. *Vibrios* that fail to penetrate the protective mucus coat are passively excreted as a result of continuous mucus degradation and replenishment. The hallmark of intestinal colonization is adherence, multiplication, and microcolony formation along the villi and crypts (Fig 3C and 3D) [72]. Conditions in the villi (low bile, high bicarbonate) are less favorable for development of mature biofilms. Thus, we suggest that

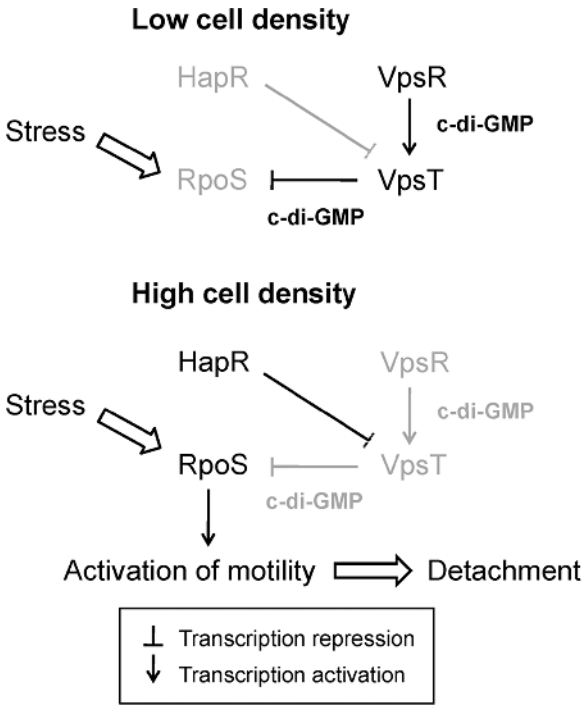

Fig 2. Model for quorum sensing and RpoS-dependent activation of motility and detachment. In a low cell density population, the regulator HapR is not expressed, the c-di-GMP content is high, and VpsT silences the transcription of *rpoS*. In a high cell density population, quorum sensing and stationary phase conditions induce the expression of HapR and RpoS that lower the c-di-GMP pool and terminate transcription of *vpsT*. In the absence of VpsT, RpoS is expressed to enhance motility. Activation of motility allows *Vibrios* to detach from a sessile community and swim away toward another unspent substratum. Inactive factors under each condition are indicated by a light grey font.

doi:10.1371/journal.pntd.0004330.g002

sessile microcolonies could represent cell aggregates remaining at an early stage of the biofilm development pathway. Late in infection, conditions of high cell density and nutrient limitation result in repression of virulence gene expression and detachment [173]. Detachment involves activation of motility and HA/protease by quorum sensing and RpoS [181–184]. This step, together with erosion of the mucus layer, re-exposes planktonic cells and/or microcolonies to the bactericidal effect of the elevated bile concentration present in the intestinal lumen (Fig 3E). Resistance to bile involves (i) the RND efflux pump [185], (ii) ToxR-dependent transcription activation of outer membrane protein OmpU [186], and (iii) ToxR activation of the

Table 1. Fitness benefits of *V. cholerae* dual lifestyle during intestinal colonization

Motile lifestyle	Sessile lifestyle
Motile *Vibrios* can swim toward the protective mucus coat and attach.	*Vibrio* biofilms exhibit enhanced infectivity.
Flagellar motility could contribute to the penetration of the protective mucus gel.	Biofilms could be more resistant to mechanical clearance.
The flagellum can cooperate with pili to facilitate *Vibrio* adherence to the protective mucus layer and underlying villi.	Biofilms could be more resistant to bile and antimicrobial peptides.
Flagellar motility participates in the regulation of virulence gene expression.	Biofilm formation could result in excretion of *Vibrios* in physiological stage more resistant to environmental stressors.

doi:10.1371/journal.pntd.0004330.t001

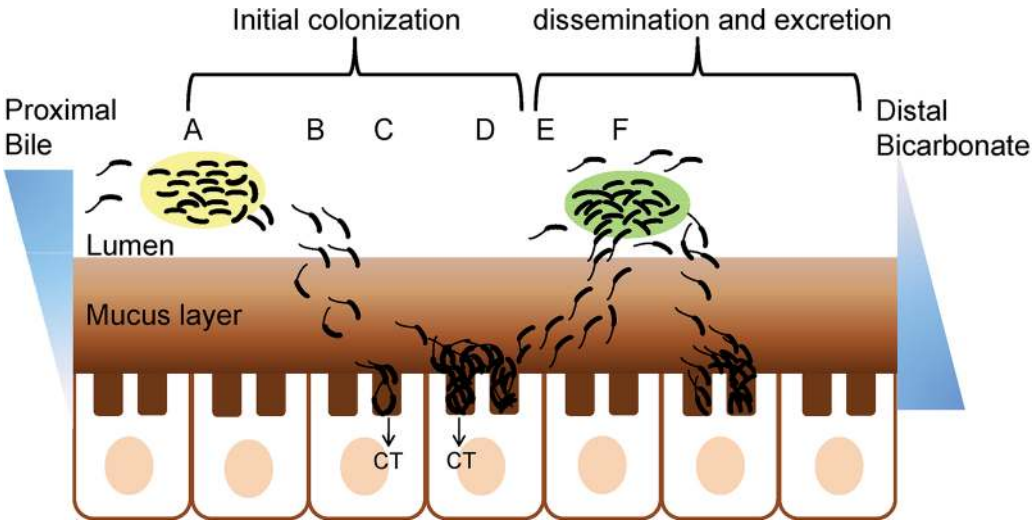

Fig 3. Model for the role of biofilm in intestinal colonization. (A) Cholera *Vibrios* can enter the small intestine as planktonic cells or embedded in a biofilm matrix, represented by a pale yellow shade. A fraction of *Vibrios* detach from the biofilm into the lumen. (B) Bile in the lumen acts as a repellent. *Vibrios* interact with the protective mucus coat and penetrate the mucus layer. (C) *Vibrios* interact with the villi. Bacterial interaction with the villi could involve iterative weak attachments that result in a more permanent adherence facilitated by TCP and other adhesins. Low bile, high bicarbonate, and cessation of motility in the proximity of the villi favor the expression of TCP and CT. (D) Expression of TCP and unknown factors promote microcolony formation along the villous axis and the crypts. (E) At high cell density, activation of HA/protease and motility by quorum sensing and RpoS promotes detachment. (F) Detached *Vibrios* are shed back into the luminal compartment. High bile concentration in the lumen enhances the c-di-GMP pool and favors biofilm formation. A fraction of detached *Vibrios* respond to bile stress by forming biofilms in vivo, indicated by *Vibrio* aggregates embedded in a pale green shade. Repetition of steps A through E spreads the infection along the small intestine. A mixture of *V*. *cholerae* planktonic cells, biofilm-like aggregates, and degraded mucus is excreted in the cholera stool.

doi:10.1371/journal.pntd.0004330.g003

LysR-family transcription activator LeuO [187], an activator of biofilm formation [34]. We suggest that detached planktonic cells or microcolonies could further aggregate into mature biofilms in the bile-rich luminal compartment (Fig 3F). Induction of *vps* and biofilm formation in the lumen could protect bacteria from bile killing [188], thereby allowing detached *Vibrios* to recolonize and disseminate along the small intestine (Fig 3). This interpretation explains the diminished intestinal colonization capacity of *vps* mutants [76]. Finally, *Vibrios* that exit the host in the form of a hyperinfective biofilm could have a higher probability of direct transmission to a secondary host (Fig 3F).

Shortcomings and Caveats

A significant amount of work on the regulation of virulence gene expression and biofilm development has been concentrated in a relatively small number of strains of serogroup O1 (classical and El Tor biotype) and O139. The focus on a reduced number of strains favors the conception of molecular models but fails to represent the broad phenotypic and genetic diversity that occurs within serogroups and biotypes. Complex phenotypes such as virulence and biofilm development integrate numerous environmental cues and can exhibit strain-specific behavior, often resulting in conflicting data. Hence, the documented regulatory connections between virulence and biofilm expression summarized above should be appreciated in the context of genetic landscapes and environment conditions that can alter the expression of mutant phenotypes. An example of genetic diversity affecting virulence and biofilm formation is quorum sensing. In this case, some O1 lineages use the quorum sensing regulator HapR, and others employ the VieA regulatory system to respond to changes in cell density [189].

We also note that our limited understanding of the nature of sessile *V*. *cholerae* communities formed during infection comes with the caveat that biofilms formed under flow conditions

in the small intestine could be structurally different from static biofilms formed in LB medium [190]. A variation of RIVET named recombination-based in-biofilm expression technology (RIBET) identified differences in the regulation of hydrodynamic versus static biofilms [191]. Interestingly, this study identified genes expressed in hydrodynamic biofilms that were also expressed late in infection [191].

In summary, though there is plentiful evidence for the formation of biofilms (or biofilm-like aggregates) during infection, the structure and regulation of these sessile communities remain largely unexplored.

Key Learning Points

- *V*. *cholerae* requires the ability to alternate between motile and biofilm lifestyles to efficiently colonize the small intestine.
- Motility and chemotaxis allow *V*. *cholerae* to move toward its niche in the small intestine and attach.
- *V*. *cholerae* can form biofilm-like structures during infection.
- *V*. *cholerae* biofilms are more resistant to stressful conditions in the host and exhibit a lower infective dose.
- Biofilm formation and dispersal during infection could enhance dissemination of *V*. *cholerae* along the small intestine and its rapid transmission to a secondary host through a fecal–oral route.

Top Five Papers

1. Faruque SM, Biswas K, Udden SM, Ahmad QS, Sack DA, Nair GB, et al. Transmissibility of cholera: *in vivo*-formed biofilms and their relationship to infectivity and persistence in the environment. Proceedings of the National Academy of Sciences of the United States of America. 2006;103(16):6350–5.
2. Schild S, Tamayo R, Nelson EJ, Qadri F, Calderwood SB, Camilli A. Genes induced late in infection increase fitness of *Vibrio cholerae* after release into the environment. Cell host & microbe. 2007;2(4):264–77.
3. Tamayo R, Patimalla B, Camilli A. Growth in a biofilm induces a hyperinfectious phenotype in *Vibrio cholerae*. Infection and immunity. 2010;78(8):3560–9.
4. Fong JC, Syed KA, Klose KE, Yildiz FH. Role of *Vibrio* polysaccharide (*vps*) genes in VPS production, biofilm formation and *Vibrio cholerae* pathogenesis. Microbiology. 2010;156(Pt 9):2757–69.
5. Millet YA, Alvarez D, Ringgaard S, von Andrian UH, Davis BM, Waldor MK. Insights into *Vibrio cholerae* intestinal colonization from monitoring fluorescently labeled bacteria. PLoS Pathog. 2014;10(10):e1004405.

References

1. Alam M, Hasan NA, Sadique A, Bhuiyan NA, Ahmed KU, Nusrin S, et al. Seasonal cholera caused by Vibrio cholerae serogroups O1 and O139 in the coastal aquatic environment of Bangladesh. Applied and environmental microbiology. 2006; 72(6):4096–104. Epub 2006/06/06. doi: 10.1128/AEM.00066-06 PMID: 16751520; PubMed Central PMCID: PMC1489596.
2. Alam M, Islam A, Bhuiyan NA, Rahim N, Hossain A, Khan GY, et al. Clonal transmission, dual peak, and off-season cholera in Bangladesh. Infection ecology & epidemiology. 2011; 1. Epub 2011/01/01. doi: 10.3402/iee.v1i0.7273 PMID: 22957115; PubMed Central PMCID: PMC3426334.
3. Colwell RR. Infectious disease and environment: cholera as a paradigm for waterborne disease. International microbiology: the official journal of the Spanish Society for Microbiology. 2004; 7(4):285–9. Epub 2005/01/25. PMID: 15666250.
4. Colwell RR. A voyage of discovery: cholera, climate and complexity. Environmental microbiology. 2002; 4(2):67–9. Epub 2002/04/26. PMID: 11972615.
5. Colwell RR. Global climate and infectious disease: the cholera paradigm. Science. 1996; 274 (5295):2025–31. Epub 1996/12/20. PMID: 8953025.
6. Islam MS, Talukder KA, Khan NH, Mahmud ZH, Rahman MZ, Nair GB, et al. Variation of toxigenic Vibrio cholerae O1 in the aquatic environment of Bangladesh and its correlation with the clinical strains. Microbiology and immunology. 2004; 48(10):773–7. Epub 2004/10/27. PMID: 15502411.
7. Lipp EK, Huq A, Colwell RR. Effects of global climate on infectious disease: the cholera model. Clinical microbiology reviews. 2002; 15(4):757–70. Epub 2002/10/05. PMID: 12364378; PubMed Central PMCID: PMC126864.
8. Lobitz B, Beck L, Huq A, Wood B, Fuchs G, Faruque AS, et al. Climate and infectious disease: use of remote sensing for detection of Vibrio cholerae by indirect measurement. Proceedings of the National Academy of Sciences of the United States of America. 2000; 97(4):1438–43. Epub 2000/03/04. PMID: 10677480; PubMed Central PMCID: PMC26452.
9. Butler SM, Nelson EJ, Chowdhury N, Faruque SM, Calderwood SB, Camilli A. Cholera stool bacteria repress chemotaxis to increase infectivity. Molecular microbiology. 2006; 60(2):417–26. Epub 2006/04/01. doi: 10.1111/j.1365-2958.2006.05096.x PMID: 16573690; PubMed Central PMCID: PMC2754204.
10. Merrell DS, Butler SM, Qadri F, Dolganov NA, Alam A, Cohen MB, et al. Host-induced epidemic spread of the cholera bacterium. Nature. 2002; 417(6889):642–5. Epub 2002/06/07. doi: 10.1038/nature00778 PMID: 12050664; PubMed Central PMCID: PMC2776822.
11. Hartley DM, Morris JG Jr., Smith DL. Hyperinfectivity: a critical element in the ability of V. cholerae to cause epidemics? PLoS medicine. 2006; 3(1):e7. Epub 2005/12/02. doi: 10.1371/journal.pmed.0030007 PMID: 16318414; PubMed Central PMCID: PMC1298942.
12. Morris JG Jr. Cholera—modern pandemic disease of ancient lineage. Emerging infectious diseases. 2011; 17(11):2099–104. Epub 2011/11/22. doi: 10.3201/eid1711.111109 PMID: 22099113; PubMed Central PMCID: PMC3310593.
13. Kaper JB, Morris JG Jr., Levine MM. Cholera. Clinical microbiology reviews. 1995; 8(1):48–86. Epub 1995/01/01. PMID: 7704895; PubMed Central PMCID: PMC172849.
14. Albert MJ. Vibrio cholerae O139 Bengal. Journal of clinical microbiology. 1994; 32(10):2345–9. Epub 1994/10/01. PMID: 7814463; PubMed Central PMCID: PMC264063.
15. Herrington DA, Hall RH, Losonsky G, Mekalanos JJ, Taylor RK, Levine MM. Toxin, toxin-coregulated pili, and the toxR regulon are essential for Vibrio cholerae pathogenesis in humans. The Journal of experimental medicine. 1988; 168(4):1487–92. Epub 1988/10/01. PMID: 2902187; PubMed Central PMCID: PMC2189073.
16. Tacket CO, Taylor RK, Losonsky G, Lim Y, Nataro JP, Kaper JB, et al. Investigation of the roles of toxin-coregulated pili and mannose-sensitive hemagglutinin pili in the pathogenesis of Vibrio cholerae O139 infection. Infection and immunity. 1998; 66(2):692–5. Epub 1998/02/07. PMID: 9453628; PubMed Central PMCID: PMC107958.
17. Thelin KH, Taylor RK. Toxin-coregulated pilus, but not mannose-sensitive hemagglutinin, is required for colonization by Vibrio cholerae O1 El Tor biotype and O139 strains. Infection and immunity. 1996; 64(7):2853–6. Epub 1996/07/01. PMID: 8698524; PubMed Central PMCID: PMC174155.
18. Waldor MK, Mekalanos JJ. Lysogenic conversion by a filamentous phage encoding cholera toxin. Science. 1996; 272(5270):1910–4. Epub 1996/06/28. PMID: 8658163.
19. Karaolis DK, Johnson JA, Bailey CC, Boedeker EC, Kaper JB, Reeves PR. A Vibrio cholerae pathogenicity island associated with epidemic and pandemic strains. Proceedings of the National Academy

of Sciences of the United States of America. 1998; 95(6):3134–9. Epub 1998/04/18. PMID: 9501228; PubMed Central PMCID: PMC19707.

20. Kojima S, Yamamoto K, Kawagishi I, Homma M. The polar flagellar motor of Vibrio cholerae is driven by an Na+ motive force. Journal of bacteriology. 1999; 181(6):1927–30. Epub 1999/03/12. PMID: 10074090; PubMed Central PMCID: PMC93596.

21. Prouty MG, Correa NE, Klose KE. The novel sigma54- and sigma28-dependent flagellar gene transcription hierarchy of Vibrio cholerae. Molecular microbiology. 2001; 39(6):1595–609. Epub 2001/03/22. PMID: 11260476.

22. Hase CC. Analysis of the role of flagellar activity in virulence gene expression in Vibrio cholerae. Microbiology. 2001; 147(Pt 4):831–7. Epub 2001/04/03. PMID: 11283279.

23. Hase CC, Mekalanos JJ. Effects of changes in membrane sodium flux on virulence gene expression in Vibrio cholerae. Proceedings of the National Academy of Sciences of the United States of America. 1999; 96(6):3183–7. Epub 1999/03/17. PMID: 10077658; PubMed Central PMCID: PMC15916.

24. Silva AJ, Leitch GJ, Camilli A, Benitez JA. Contribution of hemagglutinin/protease and motility to the pathogenesis of El Tor biotype cholera. Infection and immunity. 2006; 74(4):2072–9. Epub 2006/03/23. doi: 10.1128/IAI.74.4.2072–2079.2006 PMID: 16552036; PubMed Central PMCID: PMC1418906.

25. Syed KA, Beyhan S, Correa N, Queen J, Liu J, Peng F, et al. The Vibrio cholerae flagellar regulatory hierarchy controls expression of virulence factors. Journal of bacteriology. 2009; 191(21):6555–70. Epub 2009/09/01. doi: 10.1128/JB.00949-09 PMID: 19717600; PubMed Central PMCID: PMC2795290.

26. Wang H, Zhang L, Silva AJ, Benitez JA. A quinazoline-2,4-diamino analog suppresses Vibrio cholerae flagellar motility by interacting with motor protein PomB and induces envelope stress. Antimicrobial agents and chemotherapy. 2013; 57(8):3950–9. Epub 2013/06/05. doi: 10.1128/AAC.00473-13 PMID: 23733460; PubMed Central PMCID: PMC3719709.

27. Faruque SM, Nair GB. Molecular ecology of toxigenic Vibrio cholerae. Microbiology and immunology. 2002; 46(2):59–66. Epub 2002/04/10. PMID: 11939579.

28. Matz C, McDougald D, Moreno AM, Yung PY, Yildiz FH, Kjelleberg S. Biofilm formation and phenotypic variation enhance predation-driven persistence of Vibrio cholerae. Proceedings of the National Academy of Sciences of the United States of America. 2005; 102(46):16819–24. Epub 2005/11/04. doi: 10.1073/pnas.0505350102 PMID: 16267135; PubMed Central PMCID: PMC1283802.

29. Louis P, O'Byrne CP. Life in the gut: microbial responses to stress in the gastrointestinal tract. Science progress. 2010; 93(Pt 1):7–36. Epub 2010/03/13. PMID: 20222354.

30. Merrell DS, Tischler AD, Lee SH, Camilli A. Vibrio cholerae requires rpoS for efficient intestinal colonization. Infection and immunity. 2000; 68(12):6691–6. Epub 2000/11/18. PMID: 11083783; PubMed Central PMCID: PMC97768.

31. Kovacikova G, Skorupski K. The alternative sigma factor sigma(E) plays an important role in intestinal survival and virulence in Vibrio cholerae. Infection and immunity. 2002; 70(10):5355–62. Epub 2002/09/14. PMID: 12228259; PubMed Central PMCID: PMC128310.

32. Donlan RM, Costerton JW. Biofilms: survival mechanisms of clinically relevant microorganisms. Clinical microbiology reviews. 2002; 15(2):167–93. Epub 2002/04/05. PMID: 11932229; PubMed Central PMCID: PMC118068.

33. Hall-Stoodley L, Costerton JW, Stoodley P. Bacterial biofilms: from the natural environment to infectious diseases. Nature reviews Microbiology. 2004; 2(2):95–108. Epub 2004/03/26. doi: 10.1038/nrmicro821 PMID: 15040259.

34. Moorthy S, Watnick PI. Identification of novel stage-specific genetic requirements through whole genome transcription profiling of Vibrio cholerae biofilm development. Molecular microbiology. 2005; 57(6):1623–35. Epub 2005/09/02. doi: 10.1111/j.1365-2958.2005.04797.x PMID: 16135229; PubMed Central PMCID: PMC2600799.

35. Moorthy S, Watnick PI. Genetic evidence that the Vibrio cholerae monolayer is a distinct stage in biofilm development. Molecular microbiology. 2004; 52(2):573–87. Epub 2004/04/07. doi: 10.1111/j.1365-2958.2004.04000.x PMID: 15066042; PubMed Central PMCID: PMC2501105.

36. Liu Z, Stirling FR, Zhu J. Temporal quorum-sensing induction regulates Vibrio cholerae biofilm architecture. Infection and immunity. 2007; 75(1):122–6. Epub 2006/11/01. doi: 10.1128/IAI.01190-06 PMID: 17074850; PubMed Central PMCID: PMC1828391.

37. Wang H, Wu JH, Ayala JC, Benitez JA, Silva AJ. Interplay among cyclic diguanylate, HapR, and the general stress response regulator (RpoS) in the regulation of Vibrio cholerae hemagglutinin/protease. Journal of bacteriology. 2011; 193(23):6529–38. Epub 2011/10/04. doi: 10.1128/JB.05166-11 PMID: 21965573; PubMed Central PMCID: PMC3232884.

38. Yildiz FH, Visick KL. Vibrio biofilms: so much the same yet so different. Trends in microbiology. 2009; 17(3):109–18. Epub 2009/02/24. doi: 10.1016/j.tim.2008.12.004 PMID: 19231189; PubMed Central PMCID: PMC2729562.

39. Fong JN, Yildiz FH. Biofilm Matrix Proteins. Microbiology spectrum. 2015; 3(2). Epub 2015/06/25. doi: 10.1128/microbiolspec.MB-0004-2014 PMID: 26104709; PubMed Central PMCID: PMC4480581.

40. Teschler JK, Zamorano-Sanchez D, Utada AS, Warner CJ, Wong GC, Linington RG, et al. Living in the matrix: assembly and control of Vibrio cholerae biofilms. Nature reviews Microbiology. 2015; 13 (5):255–68. Epub 2015/04/22. doi: 10.1038/nrmicro3433 PMID: 25895940; PubMed Central PMCID: PMC4437738.

41. Tischler AD, Camilli A. Cyclic diguanylate (c-di-GMP) regulates Vibrio cholerae biofilm formation. Molecular microbiology. 2004; 53(3):857–69. Epub 2004/07/17. doi: 10.1111/j.1365-2958.2004.04155.x PMID: 15255898; PubMed Central PMCID: PMC2790424.

42. Beyhan S, Odell LS, Yildiz FH. Identification and characterization of cyclic diguanylate signaling systems controlling rugosity in Vibrio cholerae. Journal of bacteriology. 2008; 190(22):7392–405. Epub 2008/09/16. doi: 10.1128/JB.00564-08 PMID: 18790873; PubMed Central PMCID: PMC2576663.

43. Beyhan S, Yildiz FH. Smooth to rugose phase variation in Vibrio cholerae can be mediated by a single nucleotide change that targets c-di-GMP signalling pathway. Molecular microbiology. 2007; 63 (4):995–1007. Epub 2007/01/20. doi: 10.1111/j.1365-2958.2006.05568.x PMID: 17233827.

44. Fong JC, Yildiz FH. Interplay between cyclic AMP-cyclic AMP receptor protein and cyclic di-GMP signaling in Vibrio cholerae biofilm formation. Journal of bacteriology. 2008; 190(20):6646–59. Epub 2008/08/19. doi: 10.1128/JB.00466-08 PMID: 18708497; PubMed Central PMCID: PMC2566190.

45. Lim B, Beyhan S, Meir J, Yildiz FH. Cyclic-diGMP signal transduction systems in Vibrio cholerae: modulation of rugosity and biofilm formation. Molecular microbiology. 2006; 60(2):331–48. Epub 2006/04/01. doi: 10.1111/j.1365-2958.2006.05106.x PMID: 16573684.

46. Shikuma NJ, Fong JC, Yildiz FH. Cellular levels and binding of c-di-GMP control subcellular localization and activity of the Vibrio cholerae transcriptional regulator VpsT. PLoS pathogens. 2012; 8(5): e1002719. Epub 2012/06/02. doi: 10.1371/journal.ppat.1002719 PMID: 22654664; PubMed Central PMCID: PMC3359988.

47. Krasteva PV, Giglio KM, Sondermann H. Sensing the messenger: the diverse ways that bacteria signal through c-di-GMP. Protein science: a publication of the Protein Society. 2012; 21(7):929–48. Epub 2012/05/18. doi: 10.1002/pro.2093 PMID: 22593024; PubMed Central PMCID: PMC3403432.

48. Tamayo R, Pratt JT, Camilli A. Roles of cyclic diguanylate in the regulation of bacterial pathogenesis. Annual review of microbiology. 2007; 61:131–48. Epub 2007/05/08. doi: 10.1146/annurev.micro.61.080706.093426 PMID: 17480182; PubMed Central PMCID: PMC2776827.

49. Srivastava D, Hsieh ML, Khataokar A, Neiditch MB, Waters CM. Cyclic di-GMP inhibits Vibrio cholerae motility by repressing induction of transcription and inducing extracellular polysaccharide production. Molecular microbiology. 2013; 90(6):1262–76. Epub 2013/10/19. doi: 10.1111/mmi.12432 PMID: 24134710; PubMed Central PMCID: PMC3881292.

50. Yildiz FH, Dolganov NA, Schoolnik GK. VpsR, a Member of the Response Regulators of the Two-Component Regulatory Systems, Is Required for Expression of vps Biosynthesis Genes and EPS (ETr)-Associated Phenotypes in Vibrio cholerae O1 El Tor. Journal of bacteriology. 2001; 183 (5):1716–26. Epub 2001/02/13. doi: 10.1128/JB.183.5.1716–1726.2001 PMID: 11160103; PubMed Central PMCID: PMC95057.

51. Casper-Lindley C, Yildiz FH. VpsT is a transcriptional regulator required for expression of vps biosynthesis genes and the development of rugose colonial morphology in Vibrio cholerae O1 El Tor. Journal of bacteriology. 2004; 186(5):1574–8. Epub 2004/02/20. PMID: 14973043; PubMed Central PMCID: PMC344397.

52. Krasteva PV, Fong JC, Shikuma NJ, Beyhan S, Navarro MV, Yildiz FH, et al. Vibrio cholerae VpsT regulates matrix production and motility by directly sensing cyclic di-GMP. Science. 2010; 327 (5967):866–8. Epub 2010/02/13. doi: 10.1126/science.1181185 PMID: 20150502; PubMed Central PMCID: PMC2828054.

53. Pratt JT, Tamayo R, Tischler AD, Camilli A. PilZ domain proteins bind cyclic diguanylate and regulate diverse processes in Vibrio cholerae. The Journal of biological chemistry. 2007; 282(17):12860–70. Epub 2007/02/20. doi: 10.1074/jbc.M611593200 PMID: 17307739; PubMed Central PMCID: PMC2790426.

54. Yildiz FH, Schoolnik GK. Vibrio cholerae O1 El Tor: identification of a gene cluster required for the rugose colony type, exopolysaccharide production, chlorine resistance, and biofilm formation. Proceedings of the National Academy of Sciences of the United States of America. 1999; 96(7):4028–33. Epub 1999/03/31. PMID: 10097157; PubMed Central PMCID: PMC22414.

55. Fong JC, Yildiz FH. The rbmBCDEF gene cluster modulates development of rugose colony morphology and biofilm formation in Vibrio cholerae. Journal of bacteriology. 2007; 189(6):2319–30. Epub 2007/01/16. doi: 10.1128/JB.01569-06 PMID: 17220218; PubMed Central PMCID: PMC1899372.

56. Berk V, Fong JC, Dempsey GT, Develioglu ON, Zhuang X, Liphardt J, et al. Molecular architecture and assembly principles of Vibrio cholerae biofilms. Science. 2012; 337(6091):236–9. Epub 2012/07/17. doi: 10.1126/science.1222981 PMID: 22798614; PubMed Central PMCID: PMC3513368.

57. Smith DR, Maestre-Reyna M, Lee G, Gerard H, Wang AH, Watnick PI. In situ proteolysis of the Vibrio cholerae matrix protein RbmA promotes biofilm recruitment. Proceedings of the National Academy of Sciences of the United States of America. 2015. Epub 2015/08/05. doi: 10.1073/pnas.1512424112 PMID: 26240338.

58. Giglio KM, Fong JC, Yildiz FH, Sondermann H. Structural basis for biofilm formation via the Vibrio cholerae matrix protein RbmA. Journal of bacteriology. 2013; 195(14):3277–86. Epub 2013/05/21. doi: 10.1128/JB.00374-13 PMID: 23687270; PubMed Central PMCID: PMC3697633.

59. Absalon C, Van Dellen K, Watnick PI. A communal bacterial adhesin anchors biofilm and bystander cells to surfaces. PLoS pathogens. 2011; 7(8):e1002210. Epub 2011/09/09. doi: 10.1371/journal.ppat.1002210 PMID: 21901100; PubMed Central PMCID: PMC3161981.

60. Beyhan S, Tischler AD, Camilli A, Yildiz FH. Transcriptome and phenotypic responses of Vibrio cholerae to increased cyclic di-GMP level. Journal of bacteriology. 2006; 188(10):3600–13. Epub 2006/05/05. doi: 10.1128/JB.188.10.3600–3613.2006 PMID: 16672614; PubMed Central PMCID: PMC1482859.

61. Okshevsky M, Meyer RL. The role of extracellular DNA in the establishment, maintenance and perpetuation of bacterial biofilms. Critical reviews in microbiology. 2015; 41(3):341–52. Epub 2013/12/07. doi: 10.3109/1040841X.2013.841639 PMID: 24303798.

62. McDonough E, Lazinski DW, Camilli A. Identification of in vivo regulators of the Vibrio cholerae xds gene using a high-throughput genetic selection. Molecular microbiology. 2014; 92(2):302–15. Epub 2014/03/29. doi: 10.1111/mmi.12557 PMID: 24673931; PubMed Central PMCID: PMC4005888.

63. Seper A, Fengler VH, Roier S, Wolinski H, Kohlwein SD, Bishop AL, et al. Extracellular nucleases and extracellular DNA play important roles in Vibrio cholerae biofilm formation. Molecular microbiology. 2011; 82(4):1015–37. Epub 2011/10/29. doi: 10.1111/j.1365-2958.2011.07867.x PMID: 22032623; PubMed Central PMCID: PMC3212620.

64. McDonough E, Kamp H, Camilli A. Vibrio cholerae phosphatases required for the utilization of nucleotides and extracellular DNA as phosphate sources. Molecular microbiology. 2015. Epub 2015/07/16. doi: 10.1111/mmi.13128 PMID: 26175126.

65. Pratt JT, McDonough E, Camilli A. PhoB regulates motility, biofilms, and cyclic di-GMP in Vibrio cholerae. Journal of bacteriology. 2009; 191(21):6632–42. Epub 2009/09/08. doi: 10.1128/JB.00708-09 PMID: 19734314; PubMed Central PMCID: PMC2795287.

66. Sultan SZ, Silva AJ, Benitez JA. The PhoB regulatory system modulates biofilm formation and stress response in El Tor biotype Vibrio cholerae. FEMS microbiology letters. 2010; 302(1):22–31. Epub 2009/11/17. doi: 10.1111/j.1574-6968.2009.01837.x PMID: 19909344; PubMed Central PMCID: PMC2792938.

67. Haugo AJ, Watnick PI. Vibrio cholerae CytR is a repressor of biofilm development. Molecular microbiology. 2002; 45(2):471–83. Epub 2002/07/19. PMID: 12123457; PubMed Central PMCID: PMC2515492.

68. Nelson ET, Clements JD, Finkelstein RA. Vibrio cholerae adherence and colonization in experimental cholera: electron microscopic studies. Infection and immunity. 1976; 14(2):527–47. Epub 1976/08/01. PMID: 971962; PubMed Central PMCID: PMC420916.

69. Jones GW, Freter R. Adhesive properties of Vibrio cholerae: nature of the interaction with isolated rabbit brush border membranes and human erythrocytes. Infection and immunity. 1976; 14(1):240–5. Epub 1976/07/01. PMID: 985805; PubMed Central PMCID: PMC420869.

70. Yamamoto T, Kamano T, Uchimura M, Iwanaga M, Yokota T. Vibrio cholerae O1 adherence to villi and lymphoid follicle epithelium: in vitro model using formalin-treated human small intestine and correlation between adherence and cell-associated hemagglutinin levels. Infection and immunity. 1988; 56(12):3241–50. Epub 1988/12/01. PMID: 2903129; PubMed Central PMCID: PMC259731.

71. Yamamoto T, Yokota T. Electron microscopic study of Vibrio cholerae O1 adherence to the mucus coat and villus surface in the human small intestine. Infection and immunity. 1988; 56(10):2753–9. Epub 1988/10/01. PMID: 3417355; PubMed Central PMCID: PMC259640.

72. Millet YA, Alvarez D, Ringgaard S, von Andrian UH, Davis BM, Waldor MK. Insights into Vibrio cholerae intestinal colonization from monitoring fluorescently labeled bacteria. PLoS pathogens. 2014; 10(10):e1004405. Epub 2014/10/03. doi: 10.1371/journal.ppat.1004405 PMID: 25275396; PubMed Central PMCID: PMC4183697.

73. Nelson EJ, Chowdhury A, Harris JB, Begum YA, Chowdhury F, Khan AI, et al. Complexity of rice-water stool from patients with Vibrio cholerae plays a role in the transmission of infectious diarrhea. Proceedings of the National Academy of Sciences of the United States of America. 2007; 104 (48):19091–6. Epub 2007/11/21. doi: 10.1073/pnas.0706352104 PMID: 18024592; PubMed Central PMCID: PMC2141913.

74. Faruque SM, Biswas K, Udden SM, Ahmad QS, Sack DA, Nair GB, et al. Transmissibility of cholera: in vivo-formed biofilms and their relationship to infectivity and persistence in the environment. Proceedings of the National Academy of Sciences of the United States of America. 2006; 103(16):6350–5. Epub 2006/04/08. doi: 10.1073/pnas.0601277103 PMID: 16601099; PubMed Central PMCID: PMC1458881.

75. Xu Q, Dziejman M, Mekalanos JJ. Determination of the transcriptome of Vibrio cholerae during intraintestinal growth and midexponential phase in vitro. Proceedings of the National Academy of Sciences of the United States of America. 2003; 100(3):1286–91. Epub 2003/01/29. doi: 10.1073/pnas.0337479100 PMID: 12552086; PubMed Central PMCID: PMC298765.

76. Fong JC, Syed KA, Klose KE, Yildiz FH. Role of Vibrio polysaccharide (vps) genes in VPS production, biofilm formation and Vibrio cholerae pathogenesis. Microbiology. 2010; 156(Pt 9):2757–69. Epub 2010/05/15. doi: 10.1099/mic.0.040196–0 PMID: 20466768; PubMed Central PMCID: PMC3068689.

77. Watnick PI, Lauriano CM, Klose KE, Croal L, Kolter R. The absence of a flagellum leads to altered colony morphology, biofilm development and virulence in Vibrio cholerae O139. Molecular microbiology. 2001; 39(2):223–35. Epub 2001/01/03. PMID: 11136445; PubMed Central PMCID: PMC2860545.

78. Blow NS, Salomon RN, Garrity K, Reveillaud I, Kopin A, Jackson FR, et al. Vibrio cholerae infection of Drosophila melanogaster mimics the human disease cholera. PLoS pathogens. 2005; 1(1):e8. Epub 2005/10/05. doi: 10.1371/journal.ppat.0010008 PMID: 16201020; PubMed Central PMCID: PMC1238743.

79. Purdy AE, Watnick PI. Spatially selective colonization of the arthropod intestine through activation of Vibrio cholerae biofilm formation. Proceedings of the National Academy of Sciences of the United States of America. 2011; 108(49):19737–42. Epub 2011/11/23. doi: 10.1073/pnas.1111530108 PMID: 22106284; PubMed Central PMCID: PMC3241763.

80. Lauriano CM, Ghosh C, Correa NE, Klose KE. The sodium-driven flagellar motor controls exopolysaccharide expression in Vibrio cholerae. Journal of bacteriology. 2004; 186(15):4864–74. Epub 2004/07/21. doi: 10.1128/JB.186.15.4864–4874.2004 PMID: 15262923; PubMed Central PMCID: PMC451641.

81. Watnick PI, Kolter R. Steps in the development of a Vibrio cholerae El Tor biofilm. Molecular microbiology. 1999; 34(3):586–95. Epub 1999/11/17. PMID: 10564499; PubMed Central PMCID: PMC2860543.

82. Richardson K. Roles of motility and flagellar structure in pathogenicity of Vibrio cholerae: analysis of motility mutants in three animal models. Infection and immunity. 1991; 59(8):2727–36. Epub 1991/08/01. PMID: 1855990; PubMed Central PMCID: PMC258079.

83. Postnova T, Gomez-Duarte OG, Richardson K. Motility mutants of Vibrio cholerae O1 have reduced adherence in vitro to human small intestinal epithelial cells as demonstrated by ELISA. Microbiology. 1996; 142 (Pt 10):2767–76. Epub 1996/10/01. PMID: 8885392.

84. Van Dellen KL, Houot L, Watnick PI. Genetic analysis of Vibrio cholerae monolayer formation reveals a key role for DeltaPsi in the transition to permanent attachment. Journal of bacteriology. 2008; 190 (24):8185–96. Epub 2008/10/14. doi: 10.1128/JB.00948-08 PMID: 18849423; PubMed Central PMCID: PMC2593239.

85. Utada AS, Bennett RR, Fong JC, Gibiansky ML, Yildiz FH, Golestanian R, et al. Vibrio cholerae use pili and flagella synergistically to effect motility switching and conditional surface attachment. Nature communications. 2014; 5:4913. Epub 2014/09/23. doi: 10.1038/ncomms5913 PMID: 25234699.

86. Watnick PI, Fullner KJ, Kolter R. A role for the mannose-sensitive hemagglutinin in biofilm formation by Vibrio cholerae El Tor. Journal of bacteriology. 1999; 181(11):3606–9. Epub 1999/05/29. PMID: 10348878; PubMed Central PMCID: PMC93833.

87. Chiavelli DA, Marsh JW, Taylor RK. The mannose-sensitive hemagglutinin of Vibrio cholerae promotes adherence to zooplankton. Applied and environmental microbiology. 2001; 67(7):3220–5. Epub 2001/06/27. doi: 10.1128/AEM.67.7.3220–3225.2001 PMID: 11425745; PubMed Central PMCID: PMC93004.

88. Chiang SL, Taylor RK, Koomey M, Mekalanos JJ. Single amino acid substitutions in the N-terminus of Vibrio cholerae TcpA affect colonization, autoagglutination, and serum resistance. Molecular microbiology. 1995; 17(6):1133–42. Epub 1995/09/01. PMID: 8594332.

89. Jude BA, Taylor RK. The physical basis of type 4 pilus-mediated microcolony formation by Vibrio cholerae O1. Journal of structural biology. 2011; 175(1):1–9. Epub 2011/04/30. doi: 10.1016/j.jsb.2011.04.008 PMID: 21527347; PubMed Central PMCID: PMC3102138.

90. Krebs SJ, Taylor RK. Protection and attachment of Vibrio cholerae mediated by the toxin-coregulated pilus in the infant mouse model. Journal of bacteriology. 2011; 193(19):5260–70. Epub 2011/08/02. doi: 10.1128/JB.00378-11 PMID: 21804008; PubMed Central PMCID: PMC3187450.

91. Rhine JA, Taylor RK. TcpA pilin sequences and colonization requirements for O1 and O139 vibrio cholerae. Molecular microbiology. 1994; 13(6):1013–20. Epub 1994/09/01. PMID: 7854116.

92. Tischler AD, Camilli A. Cyclic diguanylate regulates Vibrio cholerae virulence gene expression. Infection and immunity. 2005; 73(9):5873–82. Epub 2005/08/23. doi: 10.1128/IAI.73.9.5873–5882.2005 PMID: 16113306; PubMed Central PMCID: PMC1231145.

93. Tischler AD, Lee SH, Camilli A. The Vibrio cholerae vieSAB locus encodes a pathway contributing to cholera toxin production. Journal of bacteriology. 2002; 184(15):4104–13. Epub 2002/07/11. PMID: 12107127; PubMed Central PMCID: PMC135224.

94. Tamayo R, Schild S, Pratt JT, Camilli A. Role of cyclic Di-GMP during el tor biotype Vibrio cholerae infection: characterization of the in vivo-induced cyclic Di-GMP phosphodiesterase CdpA. Infection and immunity. 2008; 76(4):1617–27. Epub 2008/01/30. doi: 10.1128/IAI.01337-07 PMID: 18227161; PubMed Central PMCID: PMC2292854.

95. Schild S, Tamayo R, Nelson EJ, Qadri F, Calderwood SB, Camilli A. Genes induced late in infection increase fitness of Vibrio cholerae after release into the environment. Cell host & microbe. 2007; 2 (4):264–77. Epub 2007/11/17. doi: 10.1016/j.chom.2007.09.004 PMID: 18005744; PubMed Central PMCID: PMC2169296.

96. Angelichio MJ, Spector J, Waldor MK, Camilli A. Vibrio cholerae intestinal population dynamics in the suckling mouse model of infection. Infection and immunity. 1999; 67(8):3733–9. Epub 1999/07/23. PMID: 10417131; PubMed Central PMCID: PMC96647.

97. Matson JS, Withey JH, DiRita VJ. Regulatory networks controlling Vibrio cholerae virulence gene expression. Infection and immunity. 2007; 75(12):5542–9. Epub 2007/09/19. doi: 10.1128/IAI.01094-07 PMID: 17875629; PubMed Central PMCID: PMC2168339.

98. Childers BM, Klose KE. Regulation of virulence in Vibrio cholerae: the ToxR regulon. Future microbiology. 2007; 2(3):335–44. Epub 2007/07/31. doi: 10.2217/17460913.2.3.335 PMID: 17661707.

99. Hase CC, Mekalanos JJ. TcpP protein is a positive regulator of virulence gene expression in Vibrio cholerae. Proceedings of the National Academy of Sciences of the United States of America. 1998; 95(2):730–4. Epub 1998/01/22. PMID: 9435261; PubMed Central PMCID: PMC18489.

100. Kovacikova G, Skorupski K. Overlapping binding sites for the virulence gene regulators AphA, AphB and cAMP-CRP at the Vibrio cholerae tcpPH promoter. Molecular microbiology. 2001; 41(2):393–407. Epub 2001/08/08. PMID: 11489126.

101. Miller VL, Mekalanos JJ. Genetic analysis of the cholera toxin-positive regulatory gene toxR. Journal of bacteriology. 1985; 163(2):580–5. Epub 1985/08/01. PMID: 2991197; PubMed Central PMCID: PMC219161.

102. Miller VL, DiRita VJ, Mekalanos JJ. Identification of toxS, a regulatory gene whose product enhances toxR-mediated activation of the cholera toxin promoter. Journal of bacteriology. 1989; 171(3):1288–93. Epub 1989/03/01. PMID: 2646275; PubMed Central PMCID: PMC209743.

103. DiRita VJ, Parsot C, Jander G, Mekalanos JJ. Regulatory cascade controls virulence in Vibrio cholerae. Proceedings of the National Academy of Sciences of the United States of America. 1991; 88 (12):5403–7. Epub 1991/06/15. PMID: 2052618; PubMed Central PMCID: PMC51881.

104. Lee SH, Hava DL, Waldor MK, Camilli A. Regulation and temporal expression patterns of Vibrio cholerae virulence genes during infection. Cell. 1999; 99(6):625–34. Epub 1999/12/28. PMID: 10612398.

105. Dorman CJ. H-NS, the genome sentinel. Nature reviews Microbiology. 2007; 5(2):157–61. Epub 2006/12/28. doi: 10.1038/nrmicro1598 PMID: 17191074.

106. Nye MB, Pfau JD, Skorupski K, Taylor RK. Vibrio cholerae H-NS silences virulence gene expression at multiple steps in the ToxR regulatory cascade. Journal of bacteriology. 2000; 182(15):4295–303. Epub 2000/07/14. PMID: 10894740; PubMed Central PMCID: PMC101945.

107. Yu RR, DiRita VJ. Regulation of gene expression in Vibrio cholerae by ToxT involves both antirepression and RNA polymerase stimulation. Molecular microbiology. 2002; 43(1):119–34. Epub 2002/02/19. PMID: 11849541.

108. Stonehouse E, Kovacikova G, Taylor RK, Skorupski K. Integration host factor positively regulates virulence gene expression in Vibrio cholerae. Journal of bacteriology. 2008; 190(13):4736–48. Epub 2008/05/06. doi: 10.1128/JB.00089-08 PMID: 18456804; PubMed Central PMCID: PMC2446820.

109. Stonehouse EA, Hulbert RR, Nye MB, Skorupski K, Taylor RK. H-NS binding and repression of the ctx promoter in Vibrio cholerae. Journal of bacteriology. 2011; 193(4):979–88. Epub 2010/12/21. doi: 10.1128/JB.01343-09 PMID: 21169492; PubMed Central PMCID: PMC3028689.

110. Wang H, Ayala JC, Benitez JA, Silva AJ. RNA-Seq Analysis Identifies New Genes Regulated by the Histone-Like Nucleoid Structuring Protein (H-NS) Affecting Vibrio cholerae Virulence, Stress Response and Chemotaxis. PloS one. 2015; 10(2):e0118295. Epub 2015/02/14. doi: 10.1371/journal.pone.0118295 PMID: 25679988; PubMed Central PMCID: PMC4332508.

111. Wang H, Ayala JC, Silva AJ, Benitez JA. The histone-like nucleoid structuring protein (H-NS) is a repressor of Vibrio cholerae exopolysaccharide biosynthesis (vps) genes. Applied and environmental microbiology. 2012; 78(7):2482–8. Epub 2012/01/31. doi: 10.1128/AEM.07629-11 PMID: 22287003; PubMed Central PMCID: PMC3302599.

112. Ayala JC, Wang H, Silva AJ, Benitez JA. Repression by H-NS of genes required for the biosynthesis of the Vibrio cholerae biofilm matrix is modulated by the second messenger cyclic diguanylic acid. Molecular microbiology. 2015. Epub 2015/05/20. doi: 10.1111/mmi.13058 PMID: 25982817.

113. Ayala JC, Wang H, Benitez JA, Silva AJ. RNA-Seq analysis and whole genome DNA-binding profile of the histone-like nucleoid structuring protein (H-NS). Genomics data. 2015; 5:147–50. Epub 2015/06/23. doi: 10.1016/j.gdata.2015.05.039 PMID: 26097806; PubMed Central PMCID: PMC4470426.

114. Zamorano-Sanchez D, Fong JC, Kilic S, Erill I, Yildiz FH. Identification and characterization of VpsR and VpsT binding sites in Vibrio cholerae. Journal of bacteriology. 2015; 197(7):1221–35. Epub 2015/01/28. doi: 10.1128/JB.02439-14 PMID: 25622616; PubMed Central PMCID: PMC4352665.

115. Dorman CJ. Genome architecture and global gene regulation in bacteria: making progress towards a unified model? Nature reviews Microbiology. 2013; 11(5):349–55. Epub 2013/04/04. doi: 10.1038/nrmicro3007 PMID: 23549066.

116. Hatfield GW, Benham CJ. DNA topology-mediated control of global gene expression in Escherichia coli. Annual review of genetics. 2002; 36:175–203. Epub 2002/11/14. doi: 10.1146/annurev.genet.36.032902.111815 PMID: 12429691.

117. Miller MB, Skorupski K, Lenz DH, Taylor RK, Bassler BL. Parallel quorum sensing systems converge to regulate virulence in Vibrio cholerae. Cell. 2002; 110(3):303–14. Epub 2002/08/15. PMID: 12176318.

118. Rutherford ST, van Kessel JC, Shao Y, Bassler BL. AphA and LuxR/HapR reciprocally control quorum sensing in vibrios. Genes & development. 2011; 25(4):397–408. Epub 2011/02/18. doi: 10.1101/gad.2015011 PMID: 21325136; PubMed Central PMCID: PMC3042162.

119. Lenz DH, Mok KC, Lilley BN, Kulkarni RV, Wingreen NS, Bassler BL. The small RNA chaperone Hfq and multiple small RNAs control quorum sensing in Vibrio harveyi and Vibrio cholerae. Cell. 2004; 118(1):69–82. Epub 2004/07/10. doi: 10.1016/j.cell.2004.06.009 PMID: 15242645.

120. Kovacikova G, Skorupski K. Regulation of virulence gene expression in Vibrio cholerae by quorum sensing: HapR functions at the aphA promoter. Molecular microbiology. 2002; 46(4):1135–47. Epub 2002/11/08. PMID: 12421317.

121. Yang M, Frey EM, Liu Z, Bishar R, Zhu J. The virulence transcriptional activator AphA enhances biofilm formation by Vibrio cholerae by activating expression of the biofilm regulator VpsT. Infection and immunity. 2010; 78(2):697–703. Epub 2009/11/26. doi: 10.1128/IAI.00429-09 PMID: 19933826; PubMed Central PMCID: PMC2812199.

122. Waters CM, Lu W, Rabinowitz JD, Bassler BL. Quorum sensing controls biofilm formation in Vibrio cholerae through modulation of cyclic di-GMP levels and repression of vpsT. Journal of bacteriology. 2008; 190(7):2527–36. Epub 2008/01/29. doi: 10.1128/JB.01756-07 PMID: 18223081; PubMed Central PMCID: PMC2293178.

123. Deutscher J, Francke C, Postma PW. How phosphotransferase system-related protein phosphorylation regulates carbohydrate metabolism in bacteria. Microbiology and molecular biology reviews: MMBR. 2006; 70(4):939–1031. Epub 2006/12/13. doi: 10.1128/MMBR.00024-06 PMID: 17158705; PubMed Central PMCID: PMC1698508.

124. Liang W, Pascual-Montano A, Silva AJ, Benitez JA. The cyclic AMP receptor protein modulates quorum sensing, motility and multiple genes that affect intestinal colonization in Vibrio cholerae. Microbiology. 2007; 153(Pt 9):2964–75. Epub 2007/09/05. doi: 10.1099/mic.0.2007/006668-0 PMID: 17768239.

125. Silva AJ, Benitez JA. Transcriptional regulation of Vibrio cholerae hemagglutinin/protease by the cyclic AMP receptor protein and RpoS. Journal of bacteriology. 2004; 186(19):6374–82. Epub 2004/09/18. doi: 10.1128/JB.186.19.6374–6382.2004 PMID: 15375117; PubMed Central PMCID: PMC516606.

126. Liang W, Sultan SZ, Silva AJ, Benitez JA. Cyclic AMP post-transcriptionally regulates the biosynthesis of a major bacterial autoinducer to modulate the cell density required to activate quorum sensing.

FEBS letters. 2008; 582(27):3744–50. Epub 2008/10/22. doi: 10.1016/j.febslet.2008.10.008 PMID: 18930049; PubMed Central PMCID: PMC2586060.

127. Merrell DS, Hava DL, Camilli A. Identification of novel factors involved in colonization and acid tolerance of Vibrio cholerae. Molecular microbiology. 2002; 43(6):1471–91. Epub 2002/04/16. PMID: 11952899.

128. Houot L, Chang S, Absalon C, Watnick PI. Vibrio cholerae phosphoenolpyruvate phosphotransferase system control of carbohydrate transport, biofilm formation, and colonization of the germfree mouse intestine. Infection and immunity. 2010; 78(4):1482–94. Epub 2010/02/04. doi: 10.1128/IAI.01356-09 PMID: 20123708; PubMed Central PMCID: PMC2849402.

129. Liang W, Silva AJ, Benitez JA. The cyclic AMP receptor protein modulates colonial morphology in Vibrio cholerae. Applied and environmental microbiology. 2007; 73(22):7482–7. Epub 2007/10/09. doi: 10.1128/AEM.01564-07 PMID: 17921282; PubMed Central PMCID: PMC2168207.

130. Houot L, Chang S, Pickering BS, Absalon C, Watnick PI. The phosphoenolpyruvate phosphotransferase system regulates Vibrio cholerae biofilm formation through multiple independent pathways. Journal of bacteriology. 2010; 192(12):3055–67. Epub 2010/04/20. doi: 10.1128/JB.00213-10 PMID: 20400550; PubMed Central PMCID: PMC2901703.

131. Houot L, Watnick PI. A novel role for enzyme I of the Vibrio cholerae phosphoenolpyruvate phosphotransferase system in regulation of growth in a biofilm. Journal of bacteriology. 2008; 190(1):311–20. Epub 2007/11/06. doi: 10.1128/JB.01410-07 PMID: 17981973; PubMed Central PMCID: PMC2223720.

132. Lamarche MG, Wanner BL, Crepin S, Harel J. The phosphate regulon and bacterial virulence: a regulatory network connecting phosphate homeostasis and pathogenesis. FEMS microbiology reviews. 2008; 32(3):461–73. Epub 2008/02/06. doi: 10.1111/j.1574-6976.2008.00101.x PMID: 18248418.

133. Pratt JT, Ismail AM, Camilli A. PhoB regulates both environmental and virulence gene expression in Vibrio cholerae. Molecular microbiology. 2010; 77(6):1595–605. Epub 2010/07/28. doi: 10.1111/j.1365-2958.2010.07310.x PMID: 20659293; PubMed Central PMCID: PMC2981138.

134. Sikora AE, Beyhan S, Bagdasarian M, Yildiz FH, Sandkvist M. Cell envelope perturbation induces oxidative stress and changes in iron homeostasis in Vibrio cholerae. Journal of bacteriology. 2009; 191(17):5398–408. Epub 2009/06/23. doi: 10.1128/JB.00092-09 PMID: 19542276; PubMed Central PMCID: PMC2725621.

135. Hung DT, Zhu J, Sturtevant D, Mekalanos JJ. Bile acids stimulate biofilm formation in Vibrio cholerae. Molecular microbiology. 2006; 59(1):193–201. Epub 2005/12/20. doi: 10.1111/j.1365-2958.2005.04846.x PMID: 16359328.

136. Koestler BJ, Waters CM. Bile acids and bicarbonate inversely regulate intracellular cyclic di-GMP in Vibrio cholerae. Infection and immunity. 2014; 82(7):3002–14. Epub 2014/05/07. doi: 10.1128/IAI.01664-14 PMID: 24799624; PubMed Central PMCID: PMC4097643.

137. Hay AJ, Zhu J. Host intestinal signal-promoted biofilm dispersal induces Vibrio cholerae colonization. Infection and immunity. 2015; 83(1):317–23. Epub 2014/11/05. doi: 10.1128/IAI.02617-14 PMID: 25368110; PubMed Central PMCID: PMC4288906.

138. Gupta S, Chowdhury R. Bile affects production of virulence factors and motility of Vibrio cholerae. Infection and immunity. 1997; 65(3):1131–4. Epub 1997/03/01. PMID: 9038330; PubMed Central PMCID: PMC175102.

139. Chatterjee A, Dutta PK, Chowdhury R. Effect of fatty acids and cholesterol present in bile on expression of virulence factors and motility of Vibrio cholerae. Infection and immunity. 2007; 75(4):1946–53. Epub 2007/01/31. doi: 10.1128/IAI.01435-06 PMID: 17261615; PubMed Central PMCID: PMC1865667.

140. Schuhmacher DA, Klose KE. Environmental signals modulate ToxT-dependent virulence factor expression in Vibrio cholerae. Journal of bacteriology. 1999; 181(5):1508–14. Epub 1999/02/27. PMID: 10049382; PubMed Central PMCID: PMC93540.

141. Plecha SC, Withey JH. The mechanism for inhibition of Vibrio cholerae ToxT activity by the unsaturated fatty acid components of bile. Journal of bacteriology. 2015. Epub 2015/03/04. doi: 10.1128/JB.02409-14 PMID: 25733618.

142. Abuaita BH, Withey JH. Bicarbonate Induces Vibrio cholerae virulence gene expression by enhancing ToxT activity. Infection and immunity. 2009; 77(9):4111–20. Epub 2009/07/01. doi: 10.1128/IAI.00409-09 PMID: 19564378; PubMed Central PMCID: PMC2738005.

143. Thomson JJ, Withey JH. Bicarbonate increases binding affinity of Vibrio cholerae ToxT to virulence gene promoters. Journal of bacteriology. 2014; 196(22):3872–80. Epub 2014/09/04. doi: 10.1128/JB.01824-14 PMID: 25182489; PubMed Central PMCID: PMC4248830.

144. McGuckin MA, Linden SK, Sutton P, Florin TH. Mucin dynamics and enteric pathogens. Nature reviews Microbiology. 2011; 9(4):265–78. Epub 2011/03/17. doi: 10.1038/nrmicro2538 PMID: 21407243.

145. Atuma C, Strugala V, Allen A, Holm L. The adherent gastrointestinal mucus gel layer: thickness and physical state in vivo. American journal of physiology Gastrointestinal and liver physiology. 2001; 280 (5):G922–9. Epub 2001/04/09. PMID: 11292601.

146. Klose KE. The suckling mouse model of cholera. Trends in microbiology. 2000; 8(4):189–91. Epub 2001/02/07. PMID: 10754579.

147. Ritchie JM, Rui H, Bronson RT, Waldor MK. Back to the future: studying cholera pathogenesis using infant rabbits. mBio. 2010; 1(1). Epub 2010/08/07. doi: 10.1128/mBio.00047-10 PMID: 20689747; PubMed Central PMCID: PMC2912669.

148. Cameron DE, Urbach JM, Mekalanos JJ. A defined transposon mutant library and its use in identifying motility genes in Vibrio cholerae. Proceedings of the National Academy of Sciences of the United States of America. 2008; 105(25):8736–41. Epub 2008/06/25. doi: 10.1073/pnas.0803281105 PMID: 18574146; PubMed Central PMCID: PMC2438431.

149. Chiang SL, Mekalanos JJ. Use of signature-tagged transposon mutagenesis to identify Vibrio cholerae genes critical for colonization. Molecular microbiology. 1998; 27(4):797–805. Epub 1998/03/27. PMID: 9515705.

150. Fu Y, Waldor MK, Mekalanos JJ. Tn-Seq analysis of Vibrio cholerae intestinal colonization reveals a role for T6SS-mediated antibacterial activity in the host. Cell host & microbe. 2013; 14(6):652–63. Epub 2013/12/18. doi: 10.1016/j.chom.2013.11.001 PMID: 24331463; PubMed Central PMCID: PMC3951154.

151. Mandlik A, Livny J, Robins WP, Ritchie JM, Mekalanos JJ, Waldor MK. RNA-Seq-based monitoring of infection-linked changes in Vibrio cholerae gene expression. Cell host & microbe. 2011; 10(2):165–74. Epub 2011/08/17. doi: 10.1016/j.chom.2011.07.007 PMID: 21843873; PubMed Central PMCID: PMC3166260.

152. Camilli A, Beattie DT, Mekalanos JJ. Use of genetic recombination as a reporter of gene expression. Proceedings of the National Academy of Sciences of the United States of America. 1994; 91(7):2634–8. Epub 1994/03/29. PMID: 8146167; PubMed Central PMCID: PMC43424.

153. Camilli A, Mekalanos JJ. Use of recombinase gene fusions to identify Vibrio cholerae genes induced during infection. Molecular microbiology. 1995; 18(4):671–83. Epub 1995/11/01. PMID: 8817490.

154. Lee SH, Butler SM, Camilli A. Selection for in vivo regulators of bacterial virulence. Proceedings of the National Academy of Sciences of the United States of America. 2001; 98(12):6889–94. Epub 2001/06/08. doi: 10.1073/pnas.111581598 PMID: 11391007; PubMed Central PMCID: PMC34448.

155. Lombardo MJ, Michalski J, Martinez-Wilson H, Morin C, Hilton T, Osorio CG, et al. An in vivo expression technology screen for Vibrio cholerae genes expressed in human volunteers. Proceedings of the National Academy of Sciences of the United States of America. 2007; 104(46):18229–34. Epub 2007/11/08. doi: 10.1073/pnas.0705636104 PMID: 17986616; PubMed Central PMCID: PMC2084325.

156. Taylor RK, Miller VL, Furlong DB, Mekalanos JJ. Use of phoA gene fusions to identify a pilus colonization factor coordinately regulated with cholera toxin. Proceedings of the National Academy of Sciences of the United States of America. 1987; 84(9):2833–7. Epub 1987/05/01. PMID: 2883655; PubMed Central PMCID: PMC304754.

157. Megli CJ, Yuen AS, Kolappan S, Richardson MR, Dharmasena MN, Krebs SJ, et al. Crystal structure of the Vibrio cholerae colonization factor TcpF and identification of a functional immunogenic site. Journal of molecular biology. 2011; 409(2):146–58. Epub 2011/03/29. doi: 10.1016/j.jmb.2011.03.027 PMID: 21440558; PubMed Central PMCID: PMC3098003.

158. Tamayo R, Patimalla B, Camilli A. Growth in a biofilm induces a hyperinfectious phenotype in Vibrio cholerae. Infection and immunity. 2010; 78(8):3560–9. Epub 2010/06/03. doi: 10.1128/IAI.00048-10 PMID: 20515927; PubMed Central PMCID: PMC2916270.

159. Zhu J, Mekalanos JJ. Quorum sensing-dependent biofilms enhance colonization in Vibrio cholerae. Developmental cell. 2003; 5(4):647–56. Epub 2003/10/11. PMID: 14536065.

160. Mudrak B, Tamayo R. The Vibrio cholerae Pst2 phosphate transport system is upregulated in biofilms and contributes to biofilm-induced hyperinfectivity. Infection and immunity. 2012; 80(5):1794–802. Epub 2012/02/23. doi: 10.1128/IAI.06277-11 PMID: 22354023; PubMed Central PMCID: PMC3347447.

161. Butler SM, Camilli A. Both chemotaxis and net motility greatly influence the infectivity of Vibrio cholerae. Proceedings of the National Academy of Sciences of the United States of America. 2004; 101 (14):5018–23. Epub 2004/03/24. doi: 10.1073/pnas.0308052101 PMID: 15037750; PubMed Central PMCID: PMC387366.

162. Butler SM, Camilli A. Going against the grain: chemotaxis and infection in Vibrio cholerae. Nature reviews Microbiology. 2005; 3(8):611–20. Epub 2005/07/14. doi: 10.1038/nrmicro1207 PMID: 16012515; PubMed Central PMCID: PMC2799996.

163. Wong E, Vaaje-Kolstad G, Ghosh A, Hurtado-Guerrero R, Konarev PV, Ibrahim AF, et al. The Vibrio cholerae colonization factor GbpA possesses a modular structure that governs binding to different host surfaces. PLoS pathogens. 2012; 8(1):e1002373. Epub 2012/01/19. doi: 10.1371/journal.ppat.1002373 PMID: 22253590; PubMed Central PMCID: PMC3257281.

164. Stauder M, Huq A, Pezzati E, Grim CJ, Ramoino P, Pane L, et al. Role of GbpA protein, an important virulence-related colonization factor, for Vibrio cholerae's survival in the aquatic environment. Environmental microbiology reports. 2012; 4(4):439–45. Epub 2013/06/14. doi: 10.1111/j.1758-2229.2012.00356.x PMID: 23760830.

165. Bhowmick R, Ghosal A, Das B, Koley H, Saha DR, Ganguly S, et al. Intestinal adherence of Vibrio cholerae involves a coordinated interaction between colonization factor GbpA and mucin. Infection and immunity. 2008; 76(11):4968–77. Epub 2008/09/04. doi: 10.1128/IAI.01615-07 PMID: 18765724; PubMed Central PMCID: PMC2573318.

166. Liu Z, Miyashiro T, Tsou A, Hsiao A, Goulian M, Zhu J. Mucosal penetration primes Vibrio cholerae for host colonization by repressing quorum sensing. Proceedings of the National Academy of Sciences of the United States of America. 2008; 105(28):9769–74. Epub 2008/07/09. doi: 10.1073/pnas.0802241105 PMID: 18606988; PubMed Central PMCID: PMC2474479.

167. Atsumi T, Maekawa Y, Yamada T, Kawagishi I, Imae Y, Homma M. Effect of viscosity on swimming by the lateral and polar flagella of Vibrio alginolyticus. Journal of bacteriology. 1996; 178(16):5024–6. Epub 1996/08/01. PMID: 8759871; PubMed Central PMCID: PMC178290.

168. Brown II, Hase CC. Flagellum-independent surface migration of Vibrio cholerae and Escherichia coli. Journal of bacteriology. 2001; 183(12):3784–90. Epub 2001/05/24. doi: 10.1128/JB.183.12.3784–3790.2001 PMID: 11371543; PubMed Central PMCID: PMC95256.

169. Hase CC, Finkelstein RA. Cloning and nucleotide sequence of the Vibrio cholerae hemagglutinin/protease (HA/protease) gene and construction of an HA/protease-negative strain. Journal of bacteriology. 1991; 173(11):3311–7. Epub 1991/06/01. PMID: 2045361; PubMed Central PMCID: PMC207942.

170. Silva AJ, Pham K, Benitez JA. Haemagglutinin/protease expression and mucin gel penetration in El Tor biotype Vibrio cholerae. Microbiology. 2003; 149(Pt 7):1883–91. Epub 2003/07/12. PMID: 12855739.

171. Liu Z, Wang Y, Liu S, Sheng Y, Rueggeberg KG, Wang H, et al. Vibrio cholerae Represses Polysaccharide Synthesis To Promote Motility in Mucosa. Infection and immunity. 2015; 83(3):1114–21. Epub 2015/01/07. doi: 10.1128/IAI.02841-14 PMID: 25561707.

172. Reguera G, Kolter R. Virulence and the environment: a novel role for Vibrio cholerae toxin-coregulated pili in biofilm formation on chitin. Journal of bacteriology. 2005; 187(10):3551–5. Epub 2005/05/04. doi: 10.1128/JB.187.10.3551–3555.2005 PMID: 15866944; PubMed Central PMCID: PMC1112007.

173. Zhu J, Miller MB, Vance RE, Dziejman M, Bassler BL, Mekalanos JJ. Quorum-sensing regulators control virulence gene expression in Vibrio cholerae. Proceedings of the National Academy of Sciences of the United States of America. 2002; 99(5):3129–34. Epub 2002/02/21. doi: 10.1073/pnas.052694299 PMID: 11854465; PubMed Central PMCID: PMC122484.

174. Finkelstein RA, Boesman-Finkelstein M, Chang Y, Hase CC. Vibrio cholerae hemagglutinin/protease, colonial variation, virulence, and detachment. Infection and immunity. 1992; 60(2):472–8. Epub 1992/02/01. PMID: 1730478; PubMed Central PMCID: PMC257651.

175. Benitez JA, Spelbrink RG, Silva A, Phillips TE, Stanley CM, Boesman-Finkelstein M, et al. Adherence of Vibrio cholerae to cultured differentiated human intestinal cells: an in vitro colonization model. Infection and immunity. 1997; 65(8):3474–7. Epub 1997/08/01. PMID: 9234816; PubMed Central PMCID: PMC175493.

176. Robert A, Silva A, Benitez JA, Rodriguez BL, Fando R, Campos J, et al. Tagging a Vibrio cholerae El Tor candidate vaccine strain by disruption of its hemagglutinin/protease gene using a novel reporter enzyme: Clostridium thermocellum endoglucanase A. Vaccine. 1996; 14(16):1517–22. Epub 1996/11/01. PMID: 9014293.

177. Jobling MG, Holmes RK. Characterization of hapR, a positive regulator of the Vibrio cholerae HA/protease gene hap, and its identification as a functional homologue of the Vibrio harveyi luxR gene. Molecular microbiology. 1997; 26(5):1023–34. Epub 1998/01/13. PMID: 9426139.

178. Finkelstein RA, Boesman-Finkelstein M, Holt P. Vibrio cholerae hemagglutinin/lectin/protease hydrolyzes fibronectin and ovomucin: F.M. Burnet revisited. Proceedings of the National Academy of

Sciences of the United States of America. 1983; 80(4):1092–5. Epub 1983/02/01. PMID: 6341990; PubMed Central PMCID: PMC393534.

179. Jude BA, Martinez RM, Skorupski K, Taylor RK. Levels of the secreted Vibrio cholerae attachment factor GbpA are modulated by quorum-sensing-induced proteolysis. Journal of bacteriology. 2009; 191(22):6911–7. Epub 2009/09/08. doi: 10.1128/JB.00747-09 PMID: 19734310; PubMed Central PMCID: PMC2772460.

180. Smith DR, Maestre-Reyna M, Lee G, Gerard H, Wang AH, Watnick PI. In situ proteolysis of the Vibrio cholerae matrix protein RbmA promotes biofilm recruitment. Proceedings of the National Academy of Sciences of the United States of America. 2015; 112(33):10491–6. Epub 2015/08/05. doi: 10.1073/pnas.1512424112 PMID: 26240338.

181. Wang H, Ayala JC, Benitez JA, Silva AJ. Interaction of the histone-like nucleoid structuring protein and the general stress response regulator RpoS at Vibrio cholerae promoters that regulate motility and hemagglutinin/protease expression. Journal of bacteriology. 2012; 194(5):1205–15. Epub 2011/12/24. doi: 10.1128/JB.05900-11 PMID: 22194453; PubMed Central PMCID: PMC3294804.

182. Nielsen AT, Dolganov NA, Otto G, Miller MC, Wu CY, Schoolnik GK. RpoS controls the Vibrio cholerae mucosal escape response. PLoS pathogens. 2006; 2(10):e109. Epub 2006/10/24. doi: 10.1371/journal.ppat.0020109 PMID: 17054394; PubMed Central PMCID: PMC1617127.

183. Wang H, Ayala JC, Benitez JA, Silva AJ. The LuxR-type regulator VpsT negatively controls the transcription of rpoS, encoding the general stress response regulator, in Vibrio cholerae biofilms. Journal of bacteriology. 2014; 196(5):1020–30. Epub 2013/12/24. doi: 10.1128/JB.00993-13 PMID: 24363348; PubMed Central PMCID: PMC3957697.

184. Silva AJ, Sultan SZ, Liang W, Benitez JA. Role of the histone-like nucleoid structuring protein in the regulation of rpoS and RpoS-dependent genes in Vibrio cholerae. Journal of bacteriology. 2008; 190 (22):7335–45. Epub 2008/09/16. doi: 10.1128/JB.00360-08 PMID: 18790865; PubMed Central PMCID: PMC2576668.

185. Bina XR, Provenzano D, Nguyen N, Bina JE. Vibrio cholerae RND family efflux systems are required for antimicrobial resistance, optimal virulence factor production, and colonization of the infant mouse small intestine. Infection and immunity. 2008; 76(8):3595–605. Epub 2008/05/21. doi: 10.1128/IAI.01620-07 PMID: 18490456; PubMed Central PMCID: PMC2493215.

186. Provenzano D, Klose KE. Altered expression of the ToxR-regulated porins OmpU and OmpT diminishes Vibrio cholerae bile resistance, virulence factor expression, and intestinal colonization. Proceedings of the National Academy of Sciences of the United States of America. 2000; 97(18):10220–4. Epub 2000/08/16. doi: 10.1073/pnas.170219997 PMID: 10944196; PubMed Central PMCID: PMC27820.

187. Ante VM, Bina XR, Howard MF, Sayeed S, Bina JE. Vibrio cholerae leuO transcription is positively regulated by ToxR and contributes to bile resistance. Journal of bacteriology. 2015. Epub 2015/08/26. doi: 10.1128/JB.00419-15 PMID: 26303831.

188. Bilecen K, Fong JC, Cheng A, Jones CJ, Zamorano-Sanchez D, Yildiz FH. Polymyxin B Resistance and Biofilm Formation in Vibrio cholerae Are Controlled by the Response Regulator CarR. Infection and immunity. 2015; 83(3):1199–209. Epub 2015/01/15. doi: 10.1128/IAI.02700-14 PMID: 25583523.

189. Hammer BK, Bassler BL. Distinct sensory pathways in Vibrio cholerae El Tor and classical biotypes modulate cyclic dimeric GMP levels to control biofilm formation. Journal of bacteriology. 2009; 191 (1):169–77. Epub 2008/10/28. doi: 10.1128/JB.01307-08 PMID: 18952786; PubMed Central PMCID: PMC2612459.

190. Muller J, Miller MC, Nielsen AT, Schoolnik GK, Spormann AM. vpsA- and luxO-independent biofilms of Vibrio cholerae. FEMS microbiology letters. 2007; 275(2):199–206. Epub 2007/08/19. doi: 10.1111/j.1574-6968.2007.00884.x PMID: 17697110.

191. Seper A, Pressler K, Kariisa A, Haid AG, Roier S, Leitner DR, et al. Identification of genes induced in Vibrio cholerae in a dynamic biofilm system. International journal of medical microbiology: IJMM. 2014; 304(5–6):749–63. Epub 2014/06/26. doi: 10.1016/j.ijmm.2014.05.011 PMID: 24962154; PubMed Central PMCID: PMC4101255.