Trends in Microbiology

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Review

Complex regulatory networks of virulence factors in *Vibrio vulnificus*

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The fulminating zoonotic pathogen *Vibrio vulnificus* is the causative agent of fatal septicemia in humans and fish, raising tremendous economic burdens in healthcare and the aquaculture industry. *V. vulnificus* exploits various virulence factors, including biofilm-related factors and exotoxins, for its persistence in nature and pathogenesis during infection. Substantial studies have found that the expression of virulence factors is coordinately regulated by numerous transcription factors that recognize the changing environments. Here, we summarize and discuss the recent discoveries of the physiological roles of virulence factors in *V. vulnificus* and their regulation by transcription factors in response to various environmental signals. This expanded understanding of molecular pathogenesis would provide novel clues to develop an effective antivirulence therapy against *V. vulnificus* infection.

Highlights

Vibrio vulnificus produces various virulence factors that enhance its fitness in changing environments as well as host cell-damaging activities and inflammatory responses during infection.

The spatiotemporal regulation of virulence factors is coordinated by numerous transcription factors that integrate diverse environmental signals such as nutrient availability, bacterial cell density, and antimicrobial agents.

Small molecules that inhibit the activity of virulence-associated transcription factors can significantly attenuate the virulence phenotypes of *V. vulnificus* without affecting its *in vitro* growth.

A comprehensive understanding of virulence regulation would allow us to determine effective control targets for the development of sustainable antivirulence therapies to combat *Vibrio* infection.

Increasing threats of the zoonotic pathogen *V. vulnificus*

V. vulnificus is a Gram-negative bacterium constituting the normal microbiota of marine and estuarine waters worldwide [1,2]. This opportunistic human pathogen proliferates in warmer months and easily accumulates in molluscan shellfish that become a source of infection. In this regard, *V. vulnificus* infection occurs through the consumption of contaminated seafood, especially oysters, or the exposure of open wounds to contaminated water or seafood, resulting in fatal primary septicemia and wound infection, respectively. The mortality rate of *V. vulnificus* infection is the highest among those of foodborne pathogens, which accounts for 95% of seafood-related deaths in the USA [3]. Accordingly, *V. vulnificus* has the highest per-case economic impact of all foodborne diseases [4]. Furthermore, *V. vulnificus* can infect some fish that subsequently develop diseases with symptoms including hemorrhagic septicemia and death [5]. Thus, *V. vulnificus* outbreaks in fish farms are responsible for substantial losses in the aquaculture industry.

Recently, the geographic distribution of *V. vulnificus* and the incidence of infection have increased, which presumably results from global warming and significant rises in seawater temperature [1,6]. Therefore, there is a growing need for developing effective control strategies that inhibit the virulence of *V. vulnificus*. To this end, a comprehensive understanding of the molecular pathogenesis of the pathogen should be pursued. This review aims to highlight the recent advances in the study of the physiological roles of virulence factors in *V. vulnificus* and their spatiotemporal regulation by multiple transcription factors.

Virulence factors of *V. vulnificus*

V. vulnificus infection is notable for the rapid onset of symptoms and can progress to fatal systemic infection within a few days. The destructive nature of infection implies that the pathogenicity of the pathogen is a multifactorial and complex phenomenon involving numerous virulence factors. In this part of the review, we cover the newly identified virulence factors related to the

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biofilm (see Glossary) formation of *V. vulnificus*. Also, recent studies on the pathological function of its exotoxins, which focus on their modes of action and *in vivo* effects on host immune and inflammatory responses, are summarized together.

Biofilm-related factors

Like many other pathogenic bacteria, *V. vulnificus* exists in a free-living planktonic state or a surface-attached biofilm state [7,8]. Bacteria in biofilms are more tolerant to environmental stresses such as nutrient limitation, antimicrobial agents, and host immune defenses than planktonic cells [9]. In addition, biofilm formation largely contributes to colonization and persistence of *V. vulnificus* in oysters. As a result, the pathogen can accumulate in oysters, which may serve as reservoirs in nature and thus the primary source of oral infection [10,11]. Given these characteristics of biofilms, the ability to form biofilms is closely linked to the transmission and pathogenesis of *V. vulnificus*.

Developmental stages of biofilm formation consist of initial surface attachment, microcolony formation, maturation into a 3D matrix, and biofilm dispersal to colonize other niches [8], which involves various virulence factors. Current studies have found that Type IV **pili** encoded by *iam* locus play an important role in the initial attachment stage [11,12]. Therefore, Iam pili facilitate biofilm formation and autoaggregation, thereby contributing to oyster colonization. The extracellular polymeric matrix of biofilms is composed of exopolysaccharides (EPSs), proteins, nucleic acids, and lipids [13]. As EPSs are the most predominant component of a biofilm matrix, *V. vulnificus* possesses three distinct loci involved in the assembly and export of EPSs: the *rbd* locus, the *brp* locus, and the EPS-III locus [14–16]. In addition, the products of the *brpLG* operon have been newly identified to promote EPS production, which is essential for robust biofilm and rugose colony formation [17]. Meanwhile, **flagellin**-homologous proteins FlaE and FlaF encoded by *flaEF* genes and a calcium-binding protein CabA encoded by a *cabABC* operon are secretory matrix proteins that function as other crucial components of the biofilm matrix [10,18,19]. They are secreted to the extracellular matrix and strengthen the biofilm matrix in association with EPSs. Last, the production of an **elastolytic protease** VvpE and a **capsular polysaccharide (CPS)** facilitates the dispersal of biofilm to colonize new niches [20,21].

Exotoxins

Upon entering the host, *V. vulnificus* expresses several exotoxins required for its severe invasiveness and tissue-damaging ability toward host cells. The multifunctional autoprocessing repeats-in-toxin (MARTX) toxin encoded by the *rtxA* gene is a key exotoxin with cytotoxic/cytopathic activities, which is a single polypeptide protein composed of repeat-containing regions at the N- and C-termini and central effector domains (Box 1) [22,23]. Once secreted, the MARTX toxin binds to host plasma membranes with the repeat regions, forms a porelike structure, and translocates its central effector domains into the host cytosol. Interestingly, the effectors are autoprocessed and liberated to the host subcellular compartments in response to host-specific molecules [24–26]. Each of the released effectors exhibits diverse cytopathic/cytotoxic activities, resulting in cytoskeleton and Golgi disruption, dysregulation of host cell signaling, and apoptotic cell death [23,27–31]. Based on its mode of action, the MARTX toxin is essential for *V. vulnificus* to improve anti-phagocytosis, colonization, and dissemination to the bloodstream and other organs, and thereby lethality in mice [32,33]. Also, recent studies further revealed that the expression of the MARTX toxin moderates immune responses of gut epithelial cells but stimulates inflammatory signaling of immune cells [34–36].

Phospholipase A_2 PlpA is the most recently discovered exotoxin that causes necrotic cell death of epithelial cells and lyses human erythrocytes of which membranes contain sufficient phosphatidylcholine [37]. Notably, PlpA cooperates sequentially with the MARTX toxin to facilitate necrotic

Glossary

Autoinducer-2 (AI-2): a furanosyl borate diester or tetrahydroxy furan that is a member of a family of signaling molecules used in quorum sensing.
Biofilm: 3D sessile communities of bacteria encased in a self-produced extracellular polymeric matrix.
cAMP: a second messenger important in many biological processes, which is synthesized by adenylate cyclase and used for intracellular signal transduction in many different organisms.
Capsular polysaccharide (CPS): extracellular polysaccharide produced by bacteria, which forms capsules firmly associated with the bacterial cell surface. CPS production contributes to the opacity of colony morphology as well as the resistance to bactericidal effects of host serum and phagocytosis by immune cells.
Cyclic di-GMP (c-di-GMP): a second messenger used in signal transduction in a wide variety of bacteria that is synthesized by diguanylate cyclases with a GGDEF domain and degraded by c-di-GMP-specific phosphodiesterases with EAL or HD-GYP domains.
Cytolysin: a secreted substance that is specifically toxic to individual cells leading to cell lysis.
Elastolytic protease: a proteolytic enzyme that hydrolyzes the peptide bonds within the protein of elastic tissue.
Flagellin: a globular protein that arranges itself in a hollow cylinder to form the filament in a bacterial flagellum.
Hemolysin: a cytolysin responsible for the disruption of erythrocytes and liberation of hemoglobins.
Hfq: an RNA-binding protein that facilitates the base pairing of bacterial small RNAs with their target mRNAs.
Peroxiredoxin: a ubiquitous family of antioxidant enzymes that are widely distributed across all kingdoms of life and catalyze the reduction of peroxides, such as H_2O_2, organic hydroperoxide, and $ONOO^-$.
Phospholipase A_2: a lipolytic enzyme that preferentially hydrolyzes the fatty acid from the *sn*-2 position of the glycerol backbone of phospholipid.
Pili: hairlike appendages found on the surface of many bacteria and archaea.
Quorum sensing: a bacterial cell-to-cell communication process in which bacteria detect diffusible signal molecules and coordinate the gene expression profile for social behavior such as biofilm formation and pathogenesis.

Box 1. The mode of action of MARTX toxins

As a large single polypeptide protein, MARTX toxins consist of variable effector domains between conserved N- and C-terminal repeat regions (Figure IA) [23]. Even different strains in the same species can possess different types of effectors, resulting in their varied pathological effects. For example, the MARTX toxin of *V. vulnificus* R99 strain induces a strong early cytokine storm in mice, which is not triggered by that of *V. vulnificus* YJ016 strain [34].

After the effector domains are translocated into the host cytoplasm, a conserved cysteine protease domain (CPD) becomes activated by binding to the eukaryotic cell-specific molecule, inositol hexakisphosphate ($InsP_6$) [24]. Then, the activated form of CPD processes the effector domain regions, resulting in the release of effectors into the host cytoplasm (Figure IB) [25,26]. The intermediate effector modules are further processed and fully activated by a makes caterpillars floppy-like domain (MCF) that interacts with ADP-ribosylation factors (ARFs) in the host cells [30,31].

Each liberated effector exhibits various cytopathic activities [23]. The MARTX toxin of clinical isolate *V. vulnificus* MO6-24/O strain contains four effector domains: the domain of unknown function in the first position (DUF1), Rho GTPase-inactivation domain (RID), alpha/beta hydrolase domain (ABH), and MCF. DUF1 interacts with prohibitin 1 in the host membrane which is predicted to be an initial receptor for the early binding of MARTX toxins. RID inactivates Rho-family GTPases that regulate cell cytoskeleton, leading to host cell rounding. ABH is a phosphatidylinositol 3-phosphate-specific phospholipase A1 inhibiting the autophagic pathway and endosomal trafficking of host cells. MCF results in apoptotic cell death and Golgi disruption. In addition to these four effectors, other clinical isolates CMCP6 and YJ016 strains have Ras/Rap1-specific endopeptidase (RRSP) that dysregulates host cell signaling. On the other hand, another biotype 3 clinical isolate BAA87 strain does not contain MCF and RRSP but possesses ExoY-like adenylate cyclase domain (ExoY) and domain X (DmX) causing intracellular accumulation of cAMP and Golgi disruption, respectively.

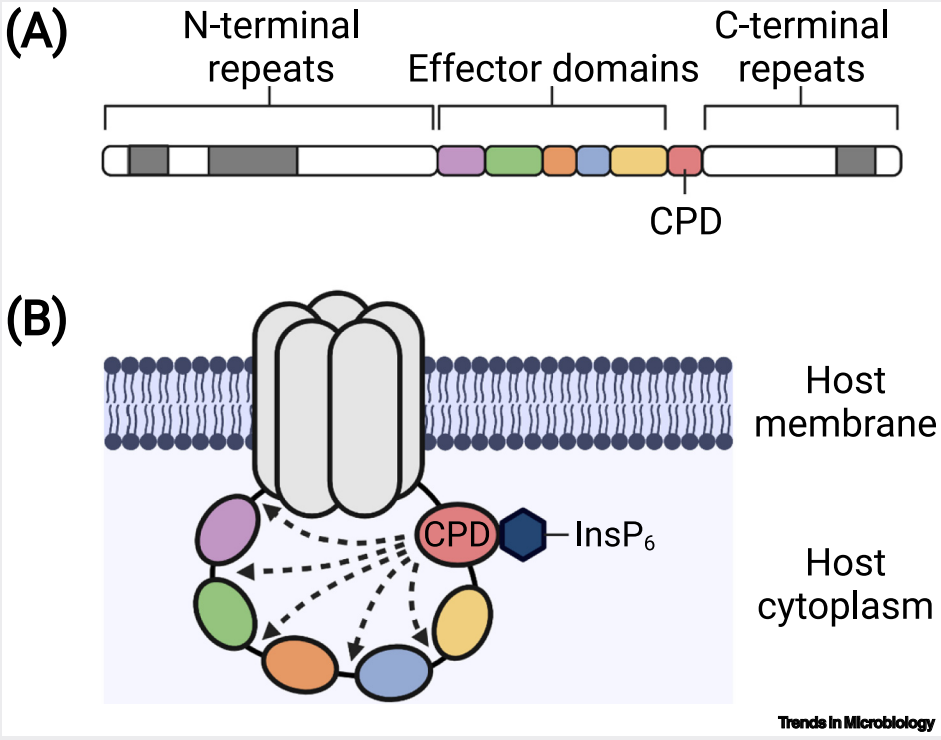

Figure I. The MARTX toxin as an effector delivery platform. (A) Schematic diagrams of a MARTX toxin. (B) Autoprocessing by $InsP_6$-activated CPD to liberate each effector domain in the host cytoplasm. Abbreviations: CPD, cysteine protease domain; $InsP_6$, inositol hexakisphosphate.

cell death by disrupting host cell blebs induced by the MARTX toxin [38]. Consistent with this, PlpA is crucial for systemic infection, liver damage, and thus lethality in mice [37]. Still, further investigations are required to elucidate the detailed mode of action of PlpA during infection.

Cytolysin/hemolysin VvhA is a pore-forming toxin conferring powerful hemolytic activity to *V. vulnificus* [39,40]. VvhA triggers apoptosis, necrotic cell death, and autophagy by dysregulation of host cell signaling, leading to host tissue damage [41,42]. Also, injection of purified VvhA induces proinflammatory cytokine and inflammatory chemokines in a mouse model [43], which presumably results in septic shock.

Elastolytic protease VvpE is an extracellular zinc metalloprotease with diverse proteolytic activities leading to host tissue damage and inflammation [44,45]. It has been reported that VvpE induces necrotic cell death via interleukin (IL)-1β production in host intestinal epithelial cells [46]. Furthermore, VvpE alters intestinal barrier function by inhibiting the expression of mucin 2, one of the major protein components of the mucus layer, and disrupting tight junctions, which contributes to the intestinal colonization of *V. vulnificus* [47,48].

Considering the significant effects of purified VvhA and VvpE, it is quite surprising that mutation of *vvhA* and *vvpE* cannot attenuate the virulence of *V. vulnificus* in mice [49], unlike that of *rtxA* and *plpA*. This might be attributable to the overwhelming potency of the MARTX toxin. Nonetheless, several pieces of evidence suggested the additive function of the MARTX toxin and VvhA for *in vivo* growth, intestinal tissue necrosis, inflammation, and dissemination of infection [32]. Thus, the cooperative or synergistic actions between exotoxins should be further examined at molecular levels to grasp their actual roles during infection. It would also be helpful to investigate at which stage of infection each exotoxin is substantially produced within the host.

Spatiotemporal regulation of virulence factors in *V. vulnificus*

To coordinate the expression of virulence factors, *V. vulnificus* takes into account a wide range of environmental signals. Thus, diverse transcription factors are exploited to integrate all the signals and produce each virulence factor at adequate levels. These complex regulatory networks enable the pathogen to effectively achieve maximum fitness in nature and within the host. Recent studies have elucidated the regulatory mechanisms of several transcription factors regarding their cognate signals and regulons. Based on these findings, we can infer the overall process by which *V. vulnificus* is transferred and establishes the pathogenesis.

Regulation of biofilm-related factors associated with *V. vulnificus* transmission

In *V. vulnificus*, biofilm and rugose colony development is promoted by the increased levels of intracellular **cyclic di-GMP (c-di-GMP)**, a universal second messenger modulating diverse bacterial behaviors [15,50]. Particularly, external calcium concentrated in oysters is known as a major signal for the increase of intracellular c-di-GMP levels [51]. A recent study suggested that BrpR, a homolog of *Vibrio cholerae* VpsR, is a master regulator of biofilm formation and colony rugosity in *V. vulnificus* [17]. BrpR is highly expressed under elevated intracellular c-di-GMP levels and subsequently regulates multiple biofilm-related regulons. BrpR activates the expression of another transcription factor *brpT*, which activates the *brp* and *cabABC* operon in a sequential cascade [52,53]. The transcriptome analysis further discovered that the *brpLG* operon is directly activated by BrpR [17]. Accordingly, BrpR enhances the production of EPS and matrix protein in response to c-di-GMP, leading to biofilm formation. Given this fact, it is tempting to speculate that *V. vulnificus* senses environmental calcium enriched on the surface of oysters and elevates the intracellular c-di-GMP levels. Then, BrpR mediates the c-di-GMP-dependent biofilm formation by coordinating the expression of its regulons, which consequently facilitates the oyster colonization and oral ingestion of *V. vulnificus* in a form of biofilms (Figure 1).

Upon entering the host, biofilms should be dispersed to planktonic cells to produce different sorts of virulence factors for intestinal colonization and disease development. It has been reported that

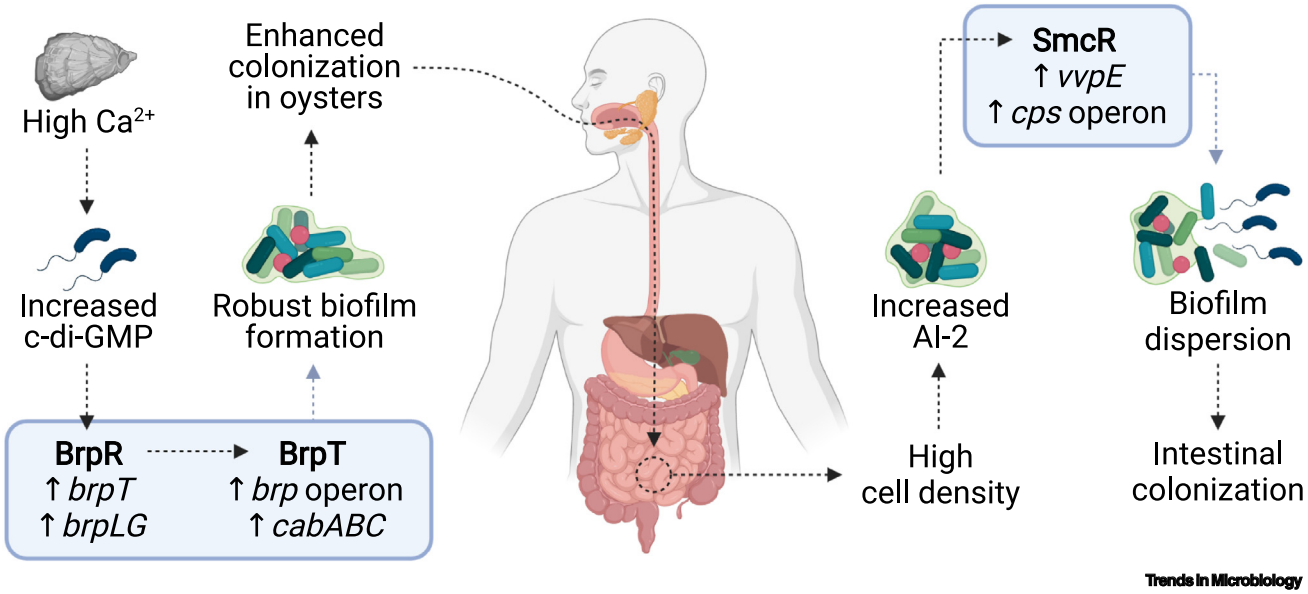

Figure 1. Model for *Vibrio vulnificus* infection in the form of biofilms. Consumption of contaminated seafood, particularly oysters, is one of the major causes of *V. vulnificus* infection. Environmental calcium is enriched on the surface of oysters, which leads to an increase in the intracellular c-di-GMP levels in *V. vulnificus*. c-di-GMP-dependent upregulation of BrpR facilitates the biofilm formation and colonization of *V. vulnificus* on the surface or tissue of oysters. Upon entering the host and when the cell density in biofilms reaches sufficiently high levels, AI-2-responsive SmcR promotes the dispersal of biofilms. Then, the detached planktonic cells alter their virulence gene expression profile for further pathogenesis such as intestinal colonization, nutrient uptakes, and exotoxin production. Abbreviations: AI-2, autoinducer-2; c-di-GMP, cyclic di-GMP.

increased bacterial cell density leads to the dispersal of *V. vulnificus* biofilms, which is governed by a **quorum-sensing** master regulator SmcR, a homolog of *Vibrio fischeri* LuxR and *V. cholerae* HapR [20,54]. *V. vulnificus* monitors its cell density with the extracellular levels of **autoinducer-2 (AI-2)**. The elevated AI-2 levels at high cell density are sensed by an inner membrane protein and subsequently increase SmcR expression through a series of signal transduction cascades (Box 2) [55]. Since SmcR activates *vvpE* and the *cps* locus, the biofilm dispersal is promoted when the cell density in biofilms sufficiently increases [20,21], contributing to the colonization of *V. vulnificus* to new niches within the host intestine (Figure 1).

Regulation of virulence factors in the early stage of infection

Once biofilm cells are dispersed in the early stage of infection, the density of planktonic *V. vulnificus* cells in host environments drastically decreases. At the same time, the pathogen becomes more sensitive to various antimicrobial stresses imposed by the host. *V. vulnificus* inevitably encounters bactericidal reactive oxygen species (ROS) and reactive nitrogen species (RNS) produced by host innate immune systems, particularly by phagocytes. Also, the host

Box 2. AI-2-mediated quorum-sensing signal transduction cascade

The quorum-sensing system in *V. vulnificus* resembles those in other *Vibrio* species such as *V. harveyi* and *V. cholerae* [54,93]. AI-2 synthesized by LuxS is considered to be a signal molecule for interspecies communication. The extracellular AI-2 levels are detected by the LuxPQ complex in bacterial inner membranes. When the AI-2 levels are insufficient at low cell density, LuxQ functions as a kinase that phosphorylates a phosphorelay protein LuxU, which in turn transfers the phosphate to the response regulator LuxO. Phosphorylated LuxO along with RpoN (σ^{54}) activates the expression of *luxT* encoding another transcription factor as well as five *qrr* genes encoding quorum regulatory RNAs. Then, LuxT and Qrr-**Hfq** complexes repress the transcription and translation of *smcR*, respectively. At high cell density, the AI-2-binding LuxPQ complex undergoes conformation changes and serves as a phosphatase that mediates the dephosphorylation of LuxO. Dephosphorylated LuxO is no longer able to repress the production of SmcR, leading to the alteration of gene expression profiles of *V. vulnificus* according to the increased cell density.

depletes free iron, an essential nutrient for *V. vulnificus* pathogenesis, by iron-sequestering systems to prevent the proliferation of the invading pathogen, which is termed 'nutritional immunity'. Iron-sulfur (Fe-S) clusters are ubiquitous cofactors that are fragile to diverse stimuli, including oxidative and nitrosative stresses and iron starvation [56]. Thus, several pathogenic bacteria use the intracellular level of the Fe-S cluster as a signal reflecting such antimicrobial conditions.

At low cell density, the expression of a [2Fe-2S]-containing transcription factor IscR is upregulated by AphA, a homolog of low cell density regulator AphA of *Vibrio harveyi* and *V. cholerae* [57]. IscR is a widely conserved sensor of intracellular Fe-S cluster levels in Gram-negative bacteria. Interestingly, *V. vulnificus* IscR coordinately regulates the expression of not only the *isc* operon for the Fe-S cluster homeostasis but also various virulence factors to cope with hostile environments [58]. IscR induces *gbpA* encoding a mucin-binding protein GbpA required for intestinal colonization of *V. vulnificus* [59]. Consistent with this, SmcR represses *gbpA* at high cell density [59], implying the need for GbpA production at the early stage of infection to colonize the host intestine. IscR also activates *prx3* encoding a 1-Cys **peroxiredoxin** Prx3 in response to oxidative and nitrosative stresses [60,61]. Prx3 can decompose both high exogenous levels of peroxides and nitric oxide, improving the survival of *V. vulnificus* against host immune systems. A recent study further revealed that IscR directly activates the expression of *vvhA* under nitrosative stress and iron starvation [62]. VvhA induced by IscR would disrupt host macrophages, epithelial cells, and erythrocytes, which contributes to immune evasion and iron acquisition. Collectively, IscR is a versatile transcription factor essential for the colonization, survival, nutrient uptake, and thereby initial establishment of *V. vulnificus* pathogenesis (Figure 2) [58].

Meanwhile, nutrient gradients within the host arise from the digestive processes or the progression of bacterial infection. The **cAMP** receptor protein (CRP) is a well-conserved global regulator of carbon and energy metabolism, which metabolically coordinates the expression of virulence factors by sensing nutrient availability [63]. A ligand for CRP is a cAMP of which intracellular levels are inversely proportional to glucose levels. Notably, CRP directly regulates the expression of four exotoxins: *rtxA* is repressed by CRP, whereas *plpA*, *vvhA*, and *vvpE* are activated [37,62,64,65]. This regulation pattern of CRP implies that the exotoxins of *V. vulnificus* may function at the different stages of infection in response to nutrient availability. For instance, the glucose levels are relatively high in the upper small intestine and bloodstream in the early stage of infection. Accordingly, the MARTX toxin would be induced by CRP in such glucose-rich environments, contributing to survival against phagocytosis and dissemination from the small intestine to other organs (Figure 2).

Regulation of virulence factors in the later stage of infection

As the bacterial infection progresses, the glucose levels gradually decrease in the ileum and bloodstream due to the absorption by enterocytes in the upper and middle small intestine or consumption by *V. vulnificus*. In the glucose-poor later stage of infection, CRP-mediated induction of PlpA, VvhA, and VvpE would lead to further inflammation and disease development (Figure 2). CRP also upregulates the expression of *hmpA* encoding a nitric oxide dioxygenase HmpA essential for survival under nitrosative stress [66]. This observation suggests that CRP coordinates alternative energy metabolism and detoxification of resulting byproducts in glucose-poor conditions.

Recently, the pathological role of another quorum-sensing molecule cyclo-(L-phenylalanine-L-proline) (cFP) has been investigated intensively [67]. *Vibrio* species, including *V. vulnificus* and *V. cholerae*, produce cFP that belongs to a family of cyclic dipeptides and affects various bacterial physiology [68]. The production of cFP reaches the maximum level in the stationary phase [69], suggesting that cFP exerts its ability in the relatively later stage of infection. In *V. vulnificus*, membrane-diffusible cFP directly binds to the periplasmic domain of the inner membrane protein ToxR [70,71]. Then,

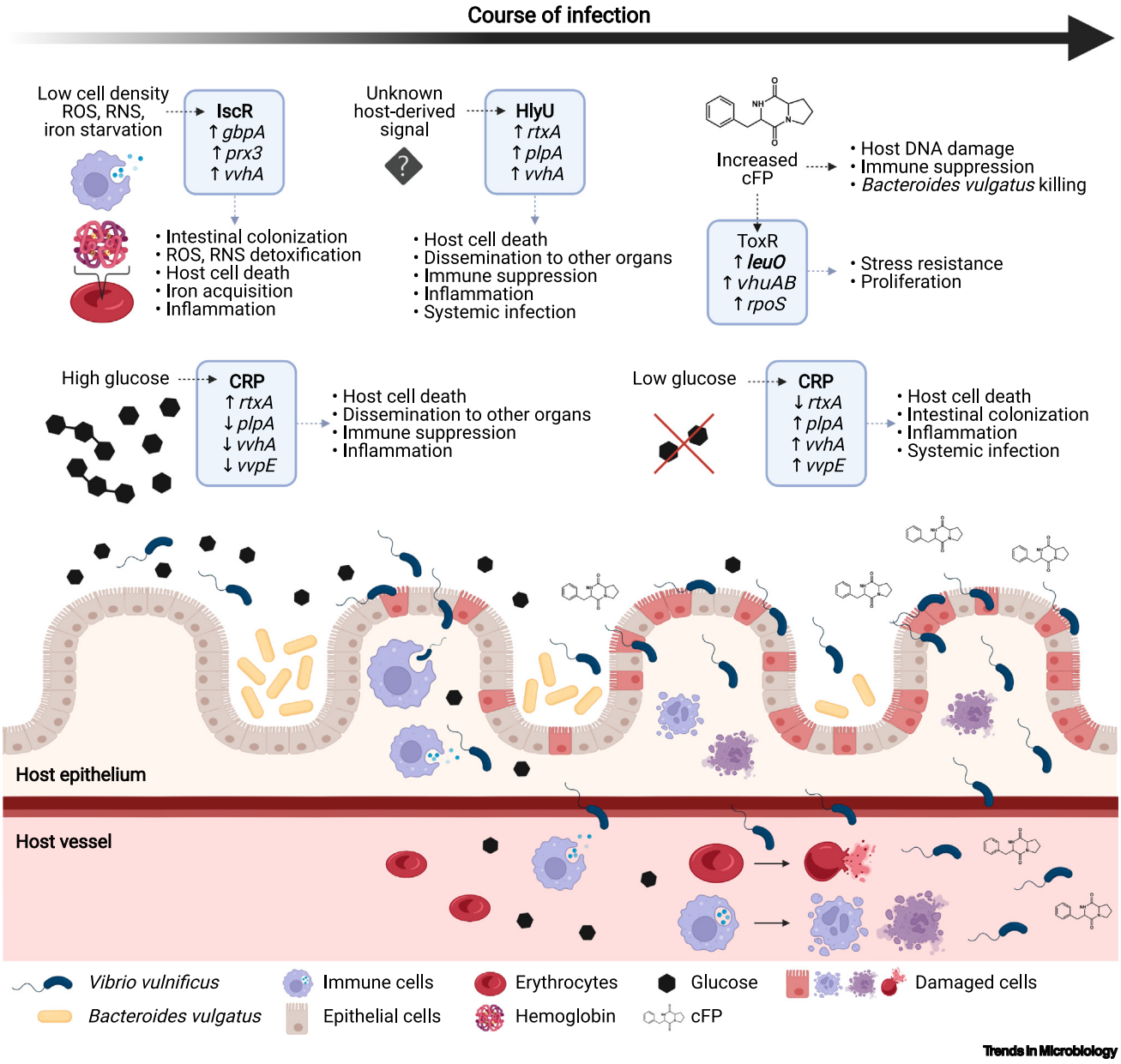

Figure 2. Model for the spatiotemporal regulation of virulence factors in *Vibrio vulnificus* during infection. *V. vulnificus* coordinately regulates the expression of virulence factors by integrating various signals during the course of infection. In the early stage of infection, the bacterial cell density of *V. vulnificus* is low and host innate immune responses become activated by the invading pathogen. Also, the glucose levels in the upper intestine and bloodstream are relatively high. IscR and CRP recognize those environmental signals and activate the expression of its adequate regulons for survival against stresses, intestinal colonization, and dissemination to other organs. In the later stage of infection, the population of *V. vulnificus* increases, and the glucose levels in the intestine and bloodstream decrease due to the absorption by enterocytes and consumption by *V. vulnificus*. Accordingly, the elevated levels of cFP enhance the fitness of *V. vulnificus* and alter the host environment to firmly establish pathogenesis. In addition, CRP coordinates the expression of exotoxins to cause further host cell damage and facilitate inflammatory responses. Although the exact host-derived signal is unknown, HlyU activates the major exotoxins and functions as a key regulator of *V. vulnificus* pathogenesis. Abbreviations: cFP, cyclo-(L-phenylalanine-L-proline); CRP, cAMP receptor protein; RNS, reactive nitrogen species; ROS, reactive oxygen species.

cFP-binding ToxR induces the expression of LeuO, a master regulator of the cFP signaling pathway [70]. LeuO activates the expression of histone-like proteins vHUα and vHUβ that positively regulate *rpoS* encoding an alternative sigma factor RpoS essential for stress responses by stabilizing its

transcript [72]. As a result, cFP modulates the regulons of ToxR, LeuO, and RpoS and thus significantly alters the gene expression profile of *V. vulnificus* involved in survival, biofilm formation, and virulence [68,70,72]. Strikingly, several studies showed that cFP acts as not only a signal molecule but also a virulence factor by itself. cFP causes DNA damage in human epithelial cells by increasing the intracellular levels of ROS and also suppresses the host immune responses by inhibiting the signaling pathway [73–75]. Moreover, cFP produced by *V. vulnificus* kills *Bacteroides vulgatus*, one of the members of gut commensals, and modifies the structure of gut microbiota during host infection (Figure 2) [76].

HlyU: a key virulence regulator of *V. vulnificus*

HlyU is one of the most significant transcription factors that cannot be omitted when describing the pathogenicity of *Vibrio* species. In *V. vulnificus*, HlyU is preferentially expressed during host infection and directly activates the expression of three exotoxins: *rtxA*, *plpA*, and *vvhA* [37,62,77,78]. Accordingly, HlyU plays a critical role in the cytotoxicity toward various host cells and lethality in mice (Figure 2). Although the expression of *hlyU* is repressed by SmcR and CRP, it seems that the indirect influence of SmcR and CRP on the expression of *rtxA* is not prominent [65,79]. Above this, other environmental signals sensed by HlyU are still not elucidated. It has been proposed that *V. cholerae* HlyU might recognize the oxidative state with its two cysteine residues [80,81]. However, further investigation is needed to clarify the external cues for HlyU.

Risk factors for *V. vulnificus* infection associated with regulation of virulence factors

Disease severity caused by *V. vulnificus* infection is largely affected by numerous risk factors, including underlying chronic diseases of patients [1,5]. Chronic liver diseases such as cirrhosis and hepatitis, hemochromatosis, diabetes, and immunocompromising diseases are well-reported underlying conditions responsible for most fatal cases. Based on the insight into the regulatory mechanisms of virulence factors, we can get some clues to explain the discrepancy in susceptibility to *V. vulnificus* infection between patients. For instance, the higher risk for those with diabetes might result from the CRP-mediated activation of the MARTX toxin caused by higher glucose levels due to diabetes. Meanwhile, chronic liver diseases typically lead to elevated iron levels in serum, which significantly accelerate bacterial *in vivo* growth and mortality from *V. vulnificus* [5,82]. Extensive studies have shown that the ferric uptake regulator (Fur) controls the expression of varied virulence factors by sensing iron availability [5,83]. However, we still cannot fully explain the exact mechanisms by which abnormal proliferation of *V. vulnificus* occurs under iron-overload conditions. Therefore, future research should aim to determine whether this phenomenon is dependent on Fur or involves other iron-responsive transcription factors.

Targeting virulence regulators: antivirulence therapy

As we discussed earlier, bacterial transcription factors fine-tune the expression of virulence factors for adaptive responses to changing environments. Thus, interfering with the function of transcription factors would prevent the pathogens from overcoming the challenges imposed by the host and developing diseases during infection. Such an approach to a so-called antivirulence therapy that disarms pathogens and facilitates their immune clearance has gained attention as a novel strategy to combat bacterial infections [84,85]. The antivirulence therapy is differentiated from traditional antibiotics in that it targets the virulence of pathogens without impeding bacterial viability. Accordingly, it is expected that the antivirulence therapy imposes low selective pressure for the emergence of antibiotic-resistant strains. Moreover, the selective attenuation of the certain pathogen would prevent unintended side effects of antibiotics such as the elimination of commensal bacteria. Recently, substantial efforts have been conducted to identify small molecules that attenuate the virulence of *V. vulnificus* by inhibiting the activity of its transcription factors [86–89].

SmcR inhibitors

Since quorum sensing controls diverse virulence traits in many pathogenic bacteria including pathogenic *Vibrio* species as well as other chronic human pathogens such as *Pseudomonas aeruginosa* and *Staphylococcus aureus*, it is considered one of the promising targets for antivirulence therapy [90]. Based on this concept, QStatin [1-(5-bromothiophene-2-sulfonyl)-1*H*-pyrazole] has been identified as a selective inhibitor of SmcR in *V. vulnificus* (Figure 3A) [88]. QStatin binds to the putative ligand-binding pocket of SmcR, resulting in the reduced regulatory activity of the protein. Notably, QStatin can disrupt the function of other LuxR homologs in other pathogenic *Vibrio* species and consequently attenuate the virulence of *V. vulnificus*, *V. harveyi*, and *Vibrio parahaemolyticus* in a brine shrimp model. Afterward, QStatin derivatives were further examined for the quorum-sensing inhibitory activity [91]. Among them, PTSP [3-phenyl-1-(thiophen-2-ylsulfonyl)-1*H*-pyrazole] exhibited the highest inhibitory activity in most *Vibrio* species, including *V. vulnificus*, *V. parahaemolyticus*, and *Vibrio campbellii* (Figure 3A). Interestingly, HapR of *V. cholerae* showed resistance against PTSP, which resulted from amino acid divergence in the ligand-binding pocket of the LuxR homologs.

Meanwhile, another SmcR inhibitory molecule, quoromycin [6-(phenylsulfonyl)nicotinonitrile] was found to hinder the DNA binding of SmcR by directly binding to SmcR (Figure 3A) [92]. The antivirulence efficacy of quoromycin has been proved in a mouse model. Quoromycin prolongs the survival of mice intragastrically infected with *V. vulnificus* biofilm. Moreover, quoromycin treatment significantly alleviates the colonization and dissemination of *V. vulnificus* within the biofilm-infected mice as well as inflammation and swelling of the small intestine.

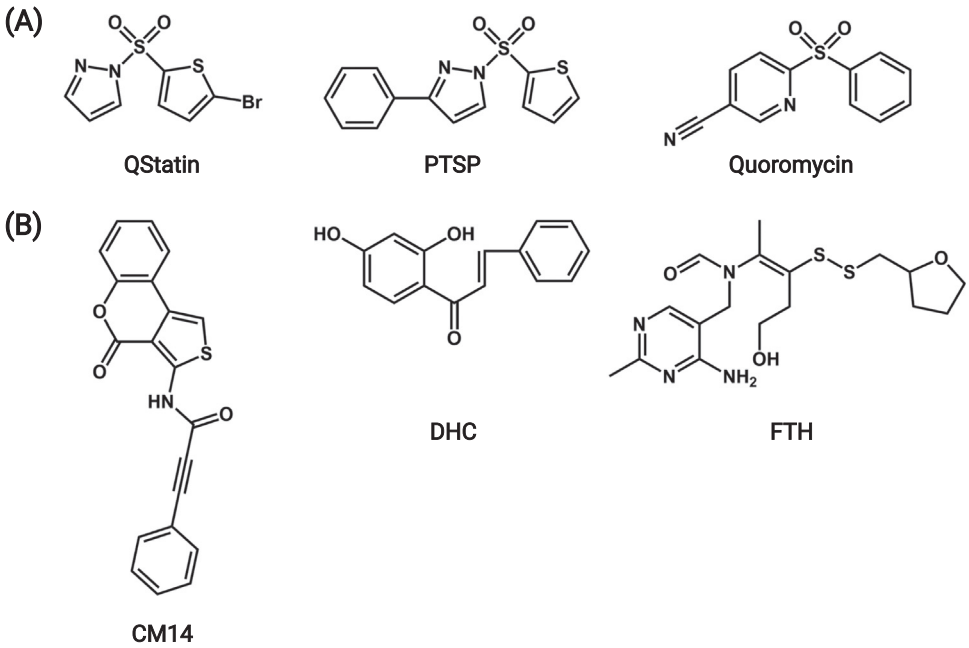

Figure 3. Small molecules that attenuate the virulence of *Vibrio vulnificus* by targeting the activity of transcription factors. (A) Chemical structures of SmcR inhibitors. QStatin, 1-(5-bromothiophene-2-sulfonyl)-1*H*-pyrazole; PTSP, 3-phenyl-1-(thiophen-2-ylsulfonyl)-1*H*-pyrazole; quoromycin, 6-(phenylsulfonyl)nicotinonitrile. (B) Chemical structures of HlyU inhibitors. CM14, *N*-(4-oxo-4H-thieno[3,4-c]chromen-3-yl)-3-phenylprop-2-ynamide; DHC, 2′,4′-dihydroxychalcone; FTH, fursultiamine hydrochloride.

HlyU inhibitors

Dysfunction of HlyU abolishes the expression of three exotoxin genes, *rtxA*, *plpA*, and *vvhA*, resulting in significant attenuation of virulence in *V. vulnificus* [37,62,65]. Since HlyU is widely conserved in other pathogenic *Vibrio* species, HlyU is another attractive target to inhibit the virulence of *Vibrio* species. Three HlyU inhibitors are currently reported: CM14 [*N*-(4-oxo-4H-thieno[3,4-c]chromen-3-yl)-3-phenylprop-2-ynamide], DHC (2′,4′-dihydroxychalcone), and FTH (fursultiamine hydrochloride) (Figure 3B) [86,87,89].

CM14 covalently modifies the cysteine residue of HlyU and interrupts its binding to target DNA [89]. Hence, CM14 reduces HlyU-dependent expression of virulence factors, leading to decrease hemolytic and host cell-damaging activities of *V. vulnificus*. Furthermore, treatment of *V. vulnificus*-infected mice with CM14 eases systemic inflammation and improves their survival rate. Intriguingly, CM14 also inhibits the virulence traits of *V. parahaemolyticus*, *Vibrio alginolyticus*, and *V. cholerae*, showing its potential as an antivirulence agent against HlyU-harboring *Vibrio* species.

Other than CM14, DHC and FTH also target the regulatory activity of HlyU and alleviate the HlyU-associated phenotypes *in vitro* [86,87]. Specifically, DHC attenuates the virulence of *V. vulnificus* in the *Galleria mellonella* model [86]. Although the detailed modes of action of DHC and FTH remain elusive, it has been shown that FTH-adapted *V. vulnificus* does not acquire resistance against FTH *in vitro* [87]. This suggests that HlyU inhibitors can be developed as an antivirulence agent with low selective pressure for the emergence of drug-resistant strains.

Concluding remarks

V. vulnificus has evolved sophisticated mechanisms to precisely regulate gene expression by integrating diverse external cues for rapid adaptive responses to changing environments. These complex regulatory networks with various transcription factors allow the pathogen to colonize in a natural habitat or within a host, survive under hostile environments, and establish successful infection. Although considerable works have been performed to understand the virulence regulation of *V. vulnificus*, our knowledge is still just the tip of the iceberg (see Outstanding questions). Above all, *in vivo* studies are required to verify that the regulatory phenomena we observed *in vitro* indeed occur during infection. In this regard, the following question is what are the key signals that primarily influence the gene expression profile of *V. vulnificus* at each stage of infection? Accordingly, the key virulence factors preferentially expressed at each stage of infection could be revealed. Furthermore, a genome-wide search for virulence-associated novel transcription factors could be another fascinating project to broaden our understanding of complicated virulence gene regulatory networks in *V. vulnificus*. Then, we should bring the individual findings together to make substantive connections between them and pathological features of *V. vulnificus* infection. Based on such efforts, the deepened insight into molecular pathogenesis could pave the way for developing more effective and sustainable antivirulence therapies against pathogenic bacteria.

Outstanding questions

How is cFP synthesized in *V. vulnificus* cells?

What are the environmental signals that switch on the regulatory activity of HlyU to upregulate the expression of three major exotoxins within the host?

Do the gene regulation phenomena, in response to external cues that we observed *in vitro,* occur *in vivo* during infection?

Among various host-derived signals, which one is the key signal that mainly affects virulence gene expression profile at each stage of infection of *V. vulnificus*?

Upon integrating all the existing environmental signals, what virulence factors are preferentially expressed and function as key players at each stage of infection?

Are there other unknown transcription factor involved in the pathogenicity of *V. vulnificus*?

Can the transcription factor-targeting small molecules identified in previous studies be clinically applied in the form of cocktails with other antivirulence agents or antibiotics?

Acknowledgments

This work was supported by the National Research Foundation of Korea, funded by the Ministry of Science, ICT, and Future Planning (2017R1E1A1A01074639 and 2021K1A3A1A20001134), and a grant (20162MFDS142) from the Ministry of Food and Drug Safety in 2022 to S.H.C. We thank all members of the Choi laboratory for their valuable discussions and technical support. The figures were created with BioRender.com.

Declaration of interests

The authors have no interests to declare.

References

1. Baker-Austin, C. and Oliver, J.D. (2018) *Vibrio vulnificus*: new insights into a deadly opportunistic pathogen. *Environ. Microbiol.* 20, 423–430
2. Baker-Austin, C. *et al.* (2018) *Vibrio* spp. infections. *Nat. Rev. Dis. Primers* 4, 8
3. Jones, M.K. and Oliver, J.D. (2009) *Vibrio vulnificus*: disease and pathogenesis. *Infect. Immun.* 77, 1723–1733
4. Hoffmann, S. and Ahn, J.W. (2021) *Updating Economic Burden of Foodborne Diseases Estimates for Inflation and Income Growth*, U.S. Department of Agriculture, Economic Research Service Published online November 18, 2021. https://ageconsearch.umn.edu/ecord/316343
5. Hernandez-Cabanyero, C. and Amaro, C. (2020) Phylogeny and life cycle of the zoonotic pathogen *Vibrio vulnificus*. *Environ. Microbiol.* 22, 4133–4148
6. Baker-Austin, C. *et al.* (2017) Non-cholera vibrios: the microbial barometer of climate change. *Trends Microbiol.* 25, 76–84
7. Yildiz, F.H. and Visick, K.L. (2009) *Vibrio* biofilms: so much the same yet so different. *Trends Microbiol.* 17, 109–118
8. Ashrafudoulla, M. *et al.* (2021) Current and future perspectives for controlling *Vibrio* biofilms in the seafood industry: a comprehensive review. *Crit. Rev. Food Sci. Nutr.* 61, 1827–1851
9. Flemming, H.C. *et al.* (2016) Biofilms: an emergent form of bacterial life. *Nat. Rev. Microbiol.* 14, 563–575
10. Park, J.H. *et al.* (2016) Role of extracellular matrix protein CabA in resistance of *Vibrio vulnificus* biofilms to decontamination strategies. *Int. J. Food Microbiol.* 236, 123–129
11. Pu, M. *et al.* (2018) A conserved tad pilus promotes *Vibrio vulnificus* oyster colonization. *Environ. Microbiol.* 20, 828–841
12. Pu, M. and Rowe-Magnus, D.A. (2018) A Tad pilus promotes the establishment and resistance of *Vibrio vulnificus* biofilms to mechanical clearance. *NPJ Biofilms Microbiomes* 4, 10
13. Flemming, H.C. and Wingender, J. (2010) The biofilm matrix. *Nat. Rev. Microbiol.* 8, 623–633
14. Kim, H.S. *et al.* (2009) Role of NtrC-regulated exopolysaccharides in the biofilm formation and pathogenic interaction of *Vibrio vulnificus*. *Mol. Microbiol.* 74, 436–453
15. Guo, Y. and Rowe-Magnus, D.A. (2010) Identification of a c-di-GMP-regulated polysaccharide locus governing stress resistance and biofilm and rugose colony formation in *Vibrio vulnificus*. *Infect. Immun.* 78, 1390–1402
16. Guo, Y. and Rowe-Magnus, D.A. (2011) Overlapping and unique contributions of two conserved polysaccharide loci in governing distinct survival phenotypes in *Vibrio vulnificus*. *Environ. Microbiol.* 13, 2888–2990
17. Hwang, S.H. *et al.* (2021) A master regulator BrpR coordinates the expression of multiple loci for robust biofilm and rugose colony development in *Vibrio vulnificus*. *Front. Microbiol.* 12, 679854
18. Park, J.H. *et al.* (2015) The *cabABC* operon essential for biofilm and rugose colony development in *Vibrio vulnificus*. *PLoS Pathog.* 11, e1005192
19. Jung, Y.C. *et al.* (2019) Role of flagellin-homologous proteins in biofilm formation by pathogenic *Vibrio* species. *mBio* 10, e01793-19
20. Kim, S.M. *et al.* (2013) LuxR homologue SmcR is essential for *Vibrio vulnificus* pathogenesis and biofilm detachment, and its expression is induced by host cells. *Infect. Immun.* 81, 3721–3730
21. Lee, K.J. *et al.* (2013) Role of capsular polysaccharide (CPS) in biofilm formation and regulation of CPS production by quorum-sensing in *Vibrio vulnificus*. *Mol. Microbiol.* 90, 841–857
22. Kim, B.S. *et al.* (2015) Distinct roles of the repeat-containing regions and effector domains of the *Vibrio vulnificus* multifunctional-autoprocessing repeats-in-toxin (MARTX) toxin. *mBio* 6, e00324-15
23. Kim, B.S. (2018) The modes of action of MARTX toxin effector domains. *Toxins (Basel)* 10, 507
24. Egerer, M. and Satchell, K.J. (2010) Inositol hexakisphosphate-induced autoprocessing of large bacterial protein toxins. *PLoS Pathog.* 6, e1000942
25. Gavin, H.E. and Satchell, K.J. (2015) MARTX toxins as effector delivery platforms. *Pathog. Dis.* 73, ftv092
26. Woida, P.J. and Satchell, K.J.F. (2018) Coordinated delivery and function of bacterial MARTX toxin effectors. *Mol. Microbiol.* 107, 133–141
27. Zhou, Y. *et al.* (2017) N^{ε}-fatty acylation of Rho GTPases by a MARTX toxin effector. *Science* 358, 528–531
28. Biancucci, M. *et al.* (2018) The bacterial Ras/Rap1 site-specific endopeptidase RRSP cleaves Ras through an atypical mechanism to disrupt Ras-ERK signaling. *Sci. Signal.* 11, eaat8335
29. Jang, S.Y. *et al.* (2018) Structural basis of inactivation of Ras and Rap1 small GTPases by Ras/Rap1-specific endopeptidase from the sepsis-causing pathogen *Vibrio vulnificus*. *J. Biol. Chem.* 293, 18110–18122
30. Lee, Y. *et al.* (2019) Makes caterpillars floppy-like effector-containing MARTX toxins require host ADP-ribosylation factor (ARF) proteins for systemic pathogenicity. *Proc. Natl. Acad. Sci. U. S. A.* 116, 18031–18040
31. Herrera, A. *et al.* (2020) N-terminal autoprocessing and acetylation of multifunctional-autoprocessing repeats-in-toxins (MARTX) Makes Caterpillars Floppy-like effector is stimulated by adenosine diphosphate (ADP)-ribosylation factor 1 in advance of Golgi fragmentation. *Cell. Microbiol.* 22, e13133
32. Jeong, H.G. and Satchell, K.J. (2012) Additive function of *Vibrio vulnificus* $MARTX_{Vv}$ and VvhA cytolysins promotes rapid growth and epithelial tissue necrosis during intestinal infection. *PLoS Pathog.* 8, e1002581
33. Gavin, H.E. *et al.* (2017) The effector domain region of the *Vibrio vulnificus* MARTX toxin confers biphasic epithelial barrier disruption and is essential for systemic spread from the intestine. *PLoS Pathog.* 13, e1006119
34. Murciano, C. *et al.* (2017) MARTX toxin in the zoonotic serovar of *Vibrio vulnificus* triggers an early cytokine storm in mice. *Front. Cell. Infect. Microbiol.* 7, 332
35. Lee, A. *et al.* (2018) *Vibrio vulnificus* RtxA is a major factor driving inflammatory T helper type 17 cell responses *in vitro* and *in vivo*. *Front. Immunol.* 9, 2095
36. Kim, B.S. *et al.* (2020) MARTX toxin-stimulated interplay between human cells and *Vibrio vulnificus*. *mSphere* 5, e00659-20
37. Jang, K.K. *et al.* (2017) Identification and characterization of *Vibrio vulnificus plpA* encoding a phospholipase A_2 essential for pathogenesis. *J. Biol. Chem.* 292, 17129–17143
38. Cho, C. *et al.* (2022) *Vibrio vulnificus* PlpA facilitates necrotic host cell death induced by the pore forming MARTX toxin. *J. Microbiol.* 60, 224–233
39. Kaus, K. *et al.* (2014) Glycan specificity of the *Vibrio vulnificus* hemolysin lectin outlines evolutionary history of membrane targeting by a toxin family. *J. Mol. Biol.* 426, 2800–2812
40. Yuan, Y. *et al.* (2020) *Vibrio vulnificus* hemolysin: biological activity, regulation of *vvhA* expression, and role in pathogenesis. *Front. Immunol.* 11, 599439
41. Lee, S.J. *et al.* (2015) *Vibrio vulnificus* VvhA induces NF-κB-dependent mitochondrial cell death via lipid raft-mediated ROS production in intestinal epithelial cells. *Cell Death Dis.* 6, 1655
42. Song, E.J. *et al.* (2016) *Vibrio vulnificus* VvhA induces autophagy-related cell death through the lipid raft-dependent c-Src/NOX signaling pathway. *Sci. Rep.* 6, 27080
43. Qin, K. *et al.* (2019) *Vibrio vulnificus* cytolysin induces inflammatory responses in RAW264.7 macrophages through calcium signaling and causes inflammation *in vivo*. *Microb. Pathog.* 137, 103789
44. Kothary, M.H. and Kreger, A.S. (1987) Purification and characterization of an elastolytic protease of *Vibrio vulnificus*. *J. Gen. Microbiol.* 133, 1783–1791
45. Miyoshi, S. (2006) *Vibrio vulnificus* infection and metalloprotease. *J. Dermatol.* 33, 589–595
46. Lee, S.J. *et al.* (2015) *Vibrio vulnificus* VvpE stimulates IL-1β production by the hypomethylation of the IL-1β promoter and NF-κB activation via lipid raft-dependent ANXA2 recruitment and reactive oxygen species signaling in intestinal epithelial cells. *J. Immunol.* 195, 2282–2293
47. Lee, S.J. *et al.* (2015) *Vibrio vulnificus* VvpE inhibits mucin 2 expression by hypermethylation via lipid raft-mediated ROS signaling in intestinal epithelial cells. *Cell Death Dis.* 6, e1787
48. Lee, S.J. *et al.* (2016) VvpE mediates the intestinal colonization of *Vibrio vulnificus* by the disruption of tight junctions. *Int. J. Med. Microbiol.* 306, 10–19

49. Fan, J.J. *et al.* (2001) Isolation and characterization of a *Vibrio vulnificus* mutant deficient in both extracellular metalloprotease and cytolysin. *Infect. Immun.* 69, 5943–5948
50. Conner, J.G. *et al.* (2017) The ins and outs of cyclic di-GMP signaling in *Vibrio cholerae*. *Curr. Opin. Microbiol.* 36, 20–29
51. Chodur, D.M. *et al.* (2018) Environmental calcium initiates a feed-forward signaling circuit that regulates biofilm formation and rugosity in *Vibrio vulnificus*. *mBio* 9, e01377-18
52. Chodur, D.M. and Rowe-Magnus, D.A. (2018) Complex control of a genomic island governing biofilm and rugose colony development in *Vibrio vulnificus*. *J. Bacteriol.* 200, e00190-18
53. Hwang, S.H. *et al.* (2020) A regulatory network controls *cabABC* expression leading to biofilm and rugose colony development in *Vibrio vulnificus*. *Front. Microbiol.* 10, 3063
54. Girard, L. (2019) Quorum sensing in *Vibrio* spp.: the complexity of multiple signalling molecules in marine and aquatic environments. *Crit. Rev. Microbiol.* 45, 451–471
55. Kim, S.Y. *et al.* (2003) Regulation of *Vibrio vulnificus* virulence by the LuxS quorum-sensing system. *Mol. Microbiol.* 48, 1647–1664
56. Miller, H.K. and Auerbuch, V. (2015) Bacterial iron-sulfur cluster sensors in mammalian pathogens. *Metallomics* 7, 943–956
57. Lim, J.G. *et al.* (2014) Low cell density regulator AphA upregulates the expression of *Vibrio vulnificus iscR* gene encoding the Fe-S cluster regulator IscR. *J. Microbiol.* 52, 413–421
58. Lim, J.G. and Choi, S.H. (2014) IscR is a global regulator essential for pathogenesis of *Vibrio vulnificus* and induced by host cells. *Infect. Immun.* 82, 569–578
59. Jang, K.K. *et al.* (2016) Regulatory characteristics of *Vibrio vulnificus gbpA* gene encoding a mucin-binding protein essential for pathogenesis. *J. Biol. Chem.* 291, 5774–5787
60. Lim, J.G. *et al.* (2014) Characterization of the *Vibrio vulnificus* 1-Cys peroxiredoxin Prx3 and regulation of its expression by the Fe-S cluster regulator IscR in response to oxidative stress and iron starvation. *J. Biol. Chem.* 289, 36263–36274
61. Ahn, J. *et al.* (2018) Crystal structure of peroxiredoxin 3 from *Vibrio vulnificus* and its implications for scavenging peroxides and nitric oxide. *IUCrJ* 5, 82–92
62. Choi, G. *et al.* (2020) The transcriptional regulator IscR integrates host-derived nitrosative stress and iron starvation in activation of the *vvhBA* operon in *Vibrio vulnificus*. *J. Biol. Chem.* 295, 5350–5361
63. Green, J. *et al.* (2014) Cyclic-AMP and bacterial cyclic-AMP receptor proteins revisited: adaptation for different ecological niches. *Curr. Opin. Microbiol.* 18, 1–7
64. Jeong, H.S. *et al.* (2003) SmcR and cyclic AMP receptor protein coactivate *Vibrio vulnificus vvpE* encoding elastase through the RpoS-dependent promoter in a synergistic manner. *J. Biol. Chem.* 278, 45072–45081
65. Lee, Z.W. *et al.* (2020) A MARTX toxin *rtxA* gene is controlled by host environmental signals through a CRP-coordinated regulatory network in *Vibrio vulnificus*. *mBio* 11, e00723-20
66. Choi, G. *et al.* (2021) A nitric oxide-responsive transcriptional regulator NsrR cooperates with Lrp and CRP to tightly control the *hmpA* gene in *Vibrio vulnificus*. *Front. Microbiol.* 12, 681196
67. Defoirdt, T. (2019) Amino acid-derived quorum sensing molecules controlling the virulence of vibrios (and beyond). *PLoS Pathog.* 15, e1007815
68. Kim, I.H. *et al.* (2013) Transcriptomic analysis of genes modulated by cyclo(L-phenylalanine-L-proline) in *Vibrio vulnificus*. *J. Microbiol. Biotechnol.* 23, 1791–1801
69. Park, D.K. *et al.* (2006) Cyclo(Phe-Pro) modulates the expression of *ompU* in *Vibrio* spp. *J. Bacteriol.* 188, 2214–2221
70. Park, N.Y. *et al.* (2019) Multi-factor regulation of the master modulator LeuO for the cyclic-(Phe-Pro) signaling pathway in *Vibrio vulnificus*. *Sci. Rep.* 9, 20135
71. Park, N.Y. *et al.* (2020) Cyclo(Phe-Pro) produced by *Vibrio* species passes through biological membranes by simple diffusion. *Appl. Microbiol. Biotechnol.* 104, 6791–6798
72. Kim, I.H. *et al.* (2018) Cyclo-(L-Phe-L-Pro), a quorum-sensing signal of *Vibrio vulnificus*, induces expression of hydroperoxidase through a ToxR-LeuO-HU-RpoS signaling pathway to confer resistance against oxidative stress. *Infect. Immun.* 86, e00932-17
73. Kim, K. *et al.* (2015) Cyclo(Phe-Pro) produced by the human pathogen *Vibrio vulnificus* inhibits host innate immune responses through the NF-κB pathway. *Infect. Immun.* 83, 1150–1161
74. Lee, K. *et al.* (2015) Cyclo(phenylalanine-proline) induces DNA damage in mammalian cells via reactive oxygen species. *J. Cell. Mol. Med.* 19, 2851–2864
75. Lee, W. *et al.* (2018) *Vibrio vulnificus* quorum-sensing molecule cyclo(Phe-Pro) inhibits RIG-I-mediated antiviral innate immunity. *Nat. Commun.* 9, 1606
76. Kim, J.A. *et al.* (2021) *Vibrio vulnificus* induces the death of a major bacterial species in the mouse gut via cyclo-Phe-Pro. *Microbiome* 9, 161
77. Kim, Y.R. *et al.* (2003) Characterization and pathogenic significance of *Vibrio vulnificus* antigens preferentially expressed in septicemic patients. *Infect. Immun.* 71, 5461–5471
78. Liu, M. *et al.* (2009) HlyU acts as an H-NS antirepressor in the regulation of the RTX toxin gene essential for the virulence of the human pathogen *Vibrio vulnificus* CMCP6. *Mol. Microbiol.* 72, 491–505
79. Shao, C.P. *et al.* (2011) Regulation of cytotoxicity by quorum-sensing signaling in *Vibrio vulnificus* is mediated by SmcR, a repressor of *hlyU*. *J. Bacteriol.* 193, 2557–2565
80. Mukherjee, D. *et al.* (2014) Crystal structure of HlyU, the hemolysin gene transcription activator, from *Vibrio cholerae* N16961 and functional implications. *Biochim. Biophys. Acta* 1844, 2346–2354
81. Mukherjee, D. *et al.* (2015) Identification of the target DNA sequence and characterization of DNA binding features of HlyU, and suggestion of a redox switch for *hlyA* expression in the human pathogen *Vibrio cholerae* from *in silico* studies. *Nucleic Acids Res.* 43, 1407–1417
82. Arezes, J. *et al.* (2015) Hepcidin-induced hypoferremia is a critical host defense mechanism against the siderophilic bacterium *Vibrio vulnificus*. *Cell Host Microbe* 17, 47–57
83. Lee, K.W. *et al.* (2022) Quorum sensing and iron-dependent coordinated control of autoinducer-2 production via small RNA RyhB in *Vibrio vulnificus*. *Sci. Rep.* 12, 831
84. Dickey, S.W. *et al.* (2017) Different drugs for bad bugs: antivirulence strategies in the age of antibiotic resistance. *Nat. Rev. Drug Discov.* 16, 457–471
85. Theuretzbacher, U. and Piddock, L.J.V. (2019) Non-traditional antibacterial therapeutic options and challenges. *Cell Host Microbe* 26, 61–72
86. Imdad, S. *et al.* (2018) Identification of 2′,4′-dihydroxychalcone as an antivirulence agent targeting HlyU, a master virulence regulator in *Vibrio vulnificus*. *Molecules* 23, 1492
87. Imdad, S. *et al.* (2018) Identification and validation of an antivirulence agent targeting HlyU-regulated virulence in *Vibrio vulnificus*. *Front. Cell. Infect. Microbiol.* 8, 152
88. Kim, B.S. *et al.* (2018) QStatin, a selective inhibitor of quorum sensing in *Vibrio* species. *mBio* 9, e02262-02217
89. Lee, Z.W. *et al.* (2019) Small-molecule inhibitor of HlyU attenuates virulence of *Vibrio* species. *Sci. Rep.* 9, 4346
90. Ellermann, M. and Sperandio, V. (2020) Bacterial signaling as an antimicrobial target. *Curr. Opin. Microbiol.* 57, 78–86
91. Newman, J.D. *et al.* (2021) Amino acid divergence in the ligand-binding pocket of *Vibrio* LuxR/HapR proteins determines the efficacy of thiophenesulfonamide inhibitors. *Mol. Microbiol.* 116, 1173–1188
92. Kim, S.M. *et al.* (2020) Phenotypic discovery of an antivirulence agent against *Vibrio vulnificus* via modulation of quorum-sensing regulator SmcR. *ACS Infect. Dis.* 6, 3076–3082
93. Papenfort, K. and Bassler, B.L. (2016) Quorum sensing signal-response systems in Gram-negative bacteria. *Nat. Rev. Microbiol.* 14, 576–588