REVIEW

Quorum sensing in vibrios: Complexity for diversification

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Abstract

N-acylhomoserine lactone-dependent quorum sensing was first discovered in two luminescent marine bacteria, *Vibrio fischeri* and *Vibrio harveyi*. The LuxI/R system of *V. fischeri* is the paradigm of Gram-negative quorum-sensing systems; however, it is not found in all vibrios. A more complex quorum-sensing regulation is found in *V. harveyi*. Three parallel systems transmit signals via phosphorelays that converge onto one regulatory protein LuxO. Components of the three systems are found only in vibrios. Of the five *Vibrio* strains analysed, the number and types of signal circuits found in each strain are diverse. The signalling systems have different regulatory responses depending on the type of association the *Vibrio* strains have with an animal host, which may reflect the diverse roles the vibrios have in structuring and maintaining microniches within the aquatic milieu. Further studies are likely to show that the diversity and complexity of the *Vibrio* quorum-sensing systems coordinate intraspecies behaviour, niche occupation, and possibly evolution.

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**Keywords:** Vibrio; Quorum sensing; Phosphorelay; Pathogenicity; Biological diversity

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Introduction

The genus *Vibrio* contains more than 50 species that are found free-living in aquatic habitats such as marine coastal waters, estuaries, sediments, and aquaculture settings as well as in association with marine organisms such as coral, fish, mollusk, seagrass, zooplankton, and shrimp (reviewed in Thompson et al., 2004). *Vibrios* associate with marine animal tissues as commensal microflora on fish mucosal surfaces, as symbionts in the light organs of fish and squid, and as pathogens causing disease in fish, coral, and crustaceans. *Vibrios* may also be bound as a biofilm to inanimate surfaces such as exoskeletons of crustaceans aiding survival during starvation and environmental stress. *Vibrios* play a role in nutrient regeneration in the aquatic milieu by taking up dissolved organic matter, producing essential polyunsaturated fatty acids needed in the aquatic food web, and degrading chitin. Some *vibrios* have a role in biodegradation of polycyclic aromatic hydrocarbons in polluted marine sediments. Among marine bacteria, *vibrios* are prolific producers of antimicrobials as well as diverse. Water properties, such as temperature, salinity, pH, sunlight, oxygen content, and nutrient availability, can change dramatically, temporally, and spatially. *Vibrios* respond with different sensitivities to environmental factors as well as in their associations with marine organisms. These differences affect their occurrence and prevalence in the aquatic environment (reviewed in Lipp et al., 2002; Tantillo et al., 2004). This immense variability of *vibrios* to cope with broad variations in the ecology is reflected in the diversity of their genomes.

*Vibrios* colonized on zooplankton or accumulated in bivalve shellfish may survive longer than free-living cells providing important bacterial reservoirs under favourable conditions. Coastal ecosystems provide ideal niches for the proliferation of *vibrios* as opposed to the open seas. Thus, aquaculture settings, which provide fish food products, are ideal reservoirs for pathogenic *vibrios* to thrive and to acquire new genes via horizontal transfer increasing the plasticity of their genomes. At least 12 *vibrios* cause disease in humans, some of which also cause disease in aquatic animals. Clinical symptoms of *Vibrio* infections are divided into three categories, gastroenteritis, primary septicemia, and wound infections. *Vibrios* are important waterborne pathogens and non-cholera *Vibrio* pathogens are considered important emerging pathogens that are transmitted by handling or consuming contaminated fish products or by exposure of an open wound to an aquatic environment (reviewed in Feldhusen, 2000; Lipp et al., 2002; Tantillo et al., 2004).

Many of these ecological encounters of *vibrios* likely utilize quorum sensing to coordinate a phenotypic response as a population. In this review, quorum-sensing systems of five *Vibrio* strains are described. The relevance of these signalling mechanisms in the orchestration of cellular responses during associations with the animal and human hosts is also discussed.

*Vibrio harveyi*

Pathogenesis

*V. harveyi* is a luminescent bacterium most notably associated with disease in cultured shrimp worldwide (Liu and Lee, 1999). In addition, eye disease and vasculitis in several fish types, skin ulcers and haemorrhagic spots near the fins and mouth of farmed sole, and necrosis with haemorrhagic spotting in tissues of packhorse rock lobster larvae are also associated with this bacterium (Austin and Austin, 1999; Diggles et al., 2000; Zorrilla et al., 2003). Siderophores are produced, and extracellular products from cultures of *V. harveyi* isolated from infected fish or shellfish are cytotoxic when injected intramuscularly into a healthy animal (Austin and Austin, 1999; Zorrilla et al., 2003). Specifically, a purified extracellular 38-kDa cysteine protease and a 100-kDa toxin T1 are toxic when injected intramuscularly into tiger prawns suggesting a possible role in the virulence of *V. harveyi* (Harris and Owens, 1999; Liu and Lee, 1999). Indirect evidence suggests that toxin production is regulated by quorum-sensing signal molecules (Manefield et al., 2000).

Quorum sensing in *V. harveyi*

Quorum sensing in *V. harveyi* utilizes three cell-signalling systems that function in parallel to regulate positively bioluminescence (Bassler et al., 1993, 1994a; Henke and Bassler, 2004a), metalloprotease (Mok et al., 2003), siderophore, and exopolysaccharide production (Lilly and Bassler, 2000) and to regulate negatively type III secretion (Henke and Bassler, 2004b) in a cell population density-dependent manner. Fig. 1 depicts a model for the *V. harveyi* quorum-sensing systems. The LuxM/N system relies on N-(3-hydroxybutanoyl)-L-homoserine lactone (3-hydroxy-C4-HSL), which is synthesized by LuxM (Cao and Meighen, 1989; Bassler et al., 1993). The LuxS/PQ system utilizes the signal molecule 3A-methyl-5,6-dihydro-furo(2,3-d)(1,3,2)dioxaborole-2,2,6,6A-tetraol (termed AI-2). The unborated AI-2 precursor is synthesized by LuxS (Surette et al., 1999; Schauder et al., 2001; Chen et al., 2002). The
CqsA/S system utilizes the signal molecule CAI-1, which is dependent on CqsA for its synthesis. The chemical structure of CAI-1 is unknown (Henke and Bassler, 2004a). These three signal molecules are distinct from each other and work synergistically in gene regulation.

The sensors for 3-hydroxy-C4-HSL, AI-2, and CAI-1, named LuxN, LuxQ, and CqsS, respectively, resemble proteins belonging to two-component signalling systems (Bassler et al., 1993, 1994a; Henke and Bassler, 2004a). Each contains a conserved histidine kinase and response regulator domain but no DNA-binding domain. At low cell population density, these synthases produce signal molecules in the cytoplasm, which either diffuse or are transported over the inner membrane (IM) and the outer membrane (OM) to the extracellular environment where they accumulate. This is not shown in the figure. Dashed single arrows indicate phosphoryl group transfers. Solid single arrows indicate positive inducing effects and solid lines with a bar at the end indicate negative inducing effects.

Vibrio harveyi

Fig. 1. Model of the V. harveyi quorum-sensing systems. For a detailed description of the model, refer to the text. At low cell population density, no signal molecules are made by LuxM, LuxS, and CqsA. At high cell population density, these synthases produce signal molecules in the cytoplasm, which either diffuse or are transported over the inner membrane (IM) and the outer membrane (OM) to the extracellular environment where they accumulate. This is not shown in the figure. Dashed single arrows indicate phosphoryl group transfers. Solid single arrows indicate positive inducing effects and solid lines with a bar at the end indicate negative inducing effects.

Vibrio cholerae

Pathogenesis

V. cholerae is a human pathogen and an example for waterborne disease (Colwell, 2004). The hallmark of cholera is a profuse “rice water” diarrhea that is a result of cholera toxin (CT) production. CT, an ADP-ribosylating toxin, is the most critical virulence factor made by V. cholerae (reviewed in Kaper et al., 1994; Dickinson and Lencer, 2003). Coregulated with CT production is the toxin-coregulated pilus (TCP), a type IV pilus essential for intestinal colonization (Taylor et al., 1987; Kirn et al., 2000). A regulatory cascade coordinates the expression of CT and TCP (reviewed in Reidl and Klose, 2002). The ctx genes, encoding CT, and the tcp gene cluster, encoding TCP, are activated directly by ToxT. toxT, in turn, is directly activated by two membrane-localized complexes, ToxR/S and TcpP/H. tcpPH is activated via synergistic binding of AphA and AphB to the tcpPH promoter. This transcriptional cascade differs in the intestinal milieu of the mouse where ctx transcription is dependent on the expression of TCP, and optimal ctx transcription requires ToxR but not TcpP (Lee et al., 1999). The bacterium first colonizes the intestinal surface utilizing TCP, and then, may receive a signal that induces full CT and TCP expression and the onset of disease. Additional factors are known to affect virulence of V. cholerae (reviewed in Reidl and Klose, 2002; Krukonis and DiRita, 2003). Motility is characterized as a virulence factor but the link to virulence is not clear. OmpU and OmpT outer membrane porins protect the bacterium against the bactericidal effects of bile in the intestines. An RTX toxin binds host cellular actin filaments leading to cytotoxicity in tissue culture cells.
and haemagglutination (HA) protease aids bacterial detachment from the intestinal tissues after infection.

**Quorum sensing in V. cholerae**

In *V. cholerae*, quorum-sensing regulation is at the top of the regulatory cascade for virulence gene expression, fine tuning the spatial and temporal expression of virulence factors to that of cell population density. *V. cholerae* contains two quorum-sensing systems similar to those of *V. harveyi*, the CqsA/S system and the LuxS/PQ system, and a third system that is not yet characterized (Miller et al., 2002). No *V. harveyi* LuxM/N system is found in the sequenced genome. Fig. 2 shows a model of the quorum-sensing systems of *V. cholerae*. The CqsA/S system and LuxS/PQ system function similarly to that of *V. harveyi* in that the sensory information is channelled in parallel through LuxU to the transcriptional activator LuxO. System 3 is believed to channel sensory information through LuxO. Thus, all three systems converge at LuxO: At low cell population density, LuxO, together with σ^32^, activates expression of four sRNAs that destabilize hapR mRNA repressing expression of HapR, a LuxR homologue (Jobling and Holmes, 1997; Lenz et al., 2004). In the absence of HapR, the ctx and tcp genes are expressed as well as polysaccharide genes (vps) required for biofilm formation (Zhu et al., 2002; Miller et al., 2002; Zhu and Mekalanos, 2003; Vance et al., 2003; Hammer and Bassler, 2003). At high cell population density, LuxO is inactivated and repression of hapR is relieved. HapR represses expression of AphA, an activator of tcpPH (Kovacikova and Skorupski, 2002). Thus, TcpP expression is repressed resulting in repression of ToxT expression and consequently repression of virulence genes. HapR also represses biofilm formation while activating expression of the HA protease.

In a model suggested by Zhu and Mekalanos (2003), biofilm formation increases acid resistance of *V. cholerae* and is thus critical for entry into the host as it passes the stomach milieu. Once in the intestinal environment, quorum-sensing signals within the biofilm repress vps, ctx, and tcp expression. The bacterium detaches from the biofilm and colonizes the intestines, where quorum-sensing signals are low and CT and TCP are expressed. As the cell population density increases in the intestinal milieu, quorum-sensing signals rise, virulence genes are repressed and HA protease is produced leading to detachment from the epithelium and exiting of the host.

**Vibrio fischeri**

**Symbiosis**

*V. fischeri* shares an exclusive symbiosis with the Hawaiian bobtail squid *Euprymna scolopes* (reviewed in Ruby, 1999; Visick and McFall-Ngai, 2000; Nyholm and McFall-Ngai, 2004). The crypts of the squid light organ are colonized by *V. fischeri* within hours after hatching. Colonization triggers morphological and developmental changes of both organisms. During embryogenesis, bacteria stimulate mucus secretion from ciliated epithelial cells at the pore openings of the light organ creating ciliar-mucus currents that increase bacterial contact with the squid. Bacteria aggregate in the mucus and in time, *V. fischeri* out-competes other bacteria and travels down the ducts to colonize the internal crypts of the light organ. After 12 h of infection, *V. fischeri* induces ciliated epithelial cell regression, enlargement of the cell lining of the crypts, and stimulates actin production in the ducts of the light organ to decrease the circumference of the ducts limiting entry to other colonizers. Eventually, the light organ matures forming tissues such as the ink sac, lens, and a thicker reflector. *V. fischeri* also undergoes changes required for entry, colonization and persistence within the crypts. After 12 h, a persistent state is acquired. The bacteria lose their flagella, reduce their cell size, reduce their growth rate after an initial burst of growth during colonization and induce bioluminescence to prevent production of toxic oxygen radicals by host enzymes. Thus, a persistent bacterial colonization requires lux
gene expression and nutrients acquired from the host tissues. The squid hosts the bacterium as an antipredation tactic. During nocturnal feeding, the squid emits light downward while modulating the intensity to mimic the moonlight so that a shadow that may be used by predators to locate their prey is not formed on the ocean floor. By venting the crypts of 90% of the bacterial population every morning, the squid controls the number of bacteria in the crypts and ensures that light is produced every evening as the bacteria increase in cell number again.

**Quorum sensing in *V. fischeri***

In contrast to *V. harveyi*, *V. fischeri* utilizes a hierarchical regulatory cascade that responds to the cell population density for sequential induction of luminescence genes, *luxICDABEG* and *luxR* (unrelated to *V. harveyi* *luxR*), and early and late colonization genes (Lupp and Ruby, 2004, 2005). A model of the regulatory cascade is shown in Fig. 3. At the bottom of the hierarchy is the LuxI/R system, the paradigm of quorum-sensing regulatory systems for many Gram-negative bacteria. This system is not found in tested strains of *V. harveyi*, *V. cholerae* and *Vibrio vulnificus*. In *V. fischeri*, the LuxI/R system regulates the *lux* genes and four non-*lux* genes of unknown function (reviewed in Miller and Bassler, 2001; Whitehead et al., 2001). At low cell population density, LuxI synthesizes only basal levels of the signal, *N-(3-oxohexanoyl)-L-homoserine lactone* (3-oxo-C6-HSL); however, as the cell numbers increase the signal molecule accumulates to a threshold level and binds LuxR, unmasking its DNA-binding domain. The LuxR-AHL complex directly activates transcription of the *luxICDABEG* genes and activates genes required for the late stage of squid colonization, resulting in exponential increase in 3-oxo-C6-HSL, light production and colonization of the inner crypts of the light organ. In this case, intercellular signals do not stimulate a phosphorelay cascade that ends in deactivation of a transcriptional repressor as for *V. harveyi* and *V. cholerae*.

At the top of the cascade are two *V. harveyi*-like quorum-sensing systems predicted to work similarly. LuxS synthesizes an AI-2 signal that is similar to that of *V. harveyi* and sensed by LuxP and LuxQ (Lupp and Ruby, 2004; Ruby et al., 2005). AinS synthase produces *N*-octanoyl-L-homoserine lactone (C8-HSL) that is sensed by AinR, a *V. harveyi* LuxN homologue (Kuo et al., 1994; Gilson et al., 1995). These two signal systems synergistically induce a phosphorelay likely via LuxO to relieve repression of LitR, a *V. harveyi* LuxR homologue (Fidopiastis et al., 2002; Miyamoto et al., 2003; Lupp and Ruby, 2004). Repression of LitR is predicted to occur via destabilization of *litR* mRNA similar to that of *luxR* in *V. harveyi*. LitR activates the LuxI/R system linking the AinS/R and LuxS/PQ systems to the LuxI/R system (Fidopiastis et al., 2002). The AinS/R system also initiates a low level of *lux* gene induction when 3-oxo-C6-HSL is limiting as the AinS signal C8-HSL binds and activates LuxR (Lupp et al., 2003). Moreover, a positive feedback loop occurs as LitR activates *ainS* (Lupp and Ruby, 2004).

This regulatory cascade induces luminescence and early and late colonization factors in a sequential manner (Visick et al., 2000; Lupp et al., 2003; Lupp and Ruby, 2004, 2005). At low cell population densities in the seawater (<10⁸ cell/ml), luminescence is repressed. At moderately high cell population densities in lab cultures and early stages of squid colonization (10⁸–10⁹ cell/ml), 3-oxo-C6-HSL is limiting. However, C8-HSL induces a low level of luminescence by binding LuxR. In the squid, the AinS/R system is not needed for luminescence. However, it regulates the expression of early colonization factors and motility (Lupp and Ruby, 2004, 2005). At high cell population densities in the squid light organ (>10¹⁰ cell/ml), 3-oxo-C6-HSL is in high amounts and the LuxI/R system induces luminescence and expression of late colonization factors. Regulation of the *lux* genes also involves additional regulatory factors not regulated by quorum sensing (reviewed in Uilitzur, 1999).

**Fig. 3.** Model of the *V. fischeri* quorum-sensing systems. For a detailed description of the model, refer to the text. The model for low cell population density is predicted to function similar to that of *V. harveyi*. Thus, only the models for intermediate and high cell population density are shown to indicate differences between species. Explanation of symbols is given in Fig. 1.
**Vibrio anguillarum**

**Pathogenesis**

*V. anguillarum* makes up part of the normal microflora of marine fish as well as of the aquatic environment (Austin and Austin, 1999). When the health or immune system of fish is compromised or when the mucosal surfaces of the fish are damaged, *V. anguillarum* causes a haemorrhagic septicaemia (vibriosis) in over 50 species of marine fish, which is associated with high rates of mortality in aquaculture. Several virulence determinants are reported (for reviews see Actis et al., 1999; Austin and Austin, 1999). A plasmid-based (pJM1) iron uptake system is essential for siderophore production during infection (reviewed in Crosa and Walsh, 2002); haem uptake is important for proliferation in fish tissues (Mazoy et al., 2003); and lipopolysaccharides play a role in serum resistance. Purified extracellular products such as haemolysins, proteases, lipases, and a neurotoxic acetylcholinesterase may play a role in the pathology of vibriosis. However, little genetic data support these suggestions. The major outer membrane protein OmpU protects against bactericidal effects of bile (Wang et al., 2003).

The intestinal tract is suggested to be the portal of entry. *V. anguillarum* adheres to the intestinal mucosa via glycosphingolipids (Olsson et al., 1996; Irie et al., 2004), colonizes, and proliferates in the intestinal tract (Olsson et al., 1998). In turbot larvae, *V. anguillarum* is transported through the intestinal epithelium by endocytosis leading to systemic infection (Grisez et al., 1996). Exopolysaccharide transport and biosynthesis are needed for microcolony formation on the skin surface and for virulence suggesting that the bacterium may enter the fish also via the skin (Milton, unpublished data). To colonize the skin or intestines, *V. anguillarum* requires chemotactic motility (O’Toole et al., 1996, 1999).

**Quorum sensing in V. anguillarum**

AHL production is a common feature of both pathogenic and environmental isolates of *V. anguillarum* suggesting that quorum sensing affects the ecology and physiology of this bacterium as well as pathogenicity (Buch et al., 2003). In *V. anguillarum*, a *V. harveyi* LuxR homologue, VanT, positively regulates extracellular protease activity, pigment production, and biofilm formation (Croxatto et al., 2002). Each of these activities may play a role in the survival of *V. anguillarum* in seawater or in the fish host. However, no direct correlation can be made yet between quorum-sensing regulation and virulence likely due to its complexity (Milton et al., 1997; Milton, unpublished data). Interestingly, a quorum-sensing inhibitor (furanone C30) decreases death due to vibriosis in rainbow trout challenged with *V. anguillarum* (Rasch et al., 2004).

Three quorum-sensing circuits are found in a pathogenic strain of *V. anguillarum* and a fourth is predicted (Fig. 4). Two systems are homologous to the LuxM/N and LuxS/PQ systems of *V. harveyi* (Milton et al., 2001; Croxatto et al., 2004). For the LuxM/N system, an AHL synthase, VanM, synthesizes both N-hexanoyl-L-homoserine lactone (C6-HSL) and N-(3-hydroxyhexanoyl)-L-homoserine lactone (3-hydroxy-C6-HSL), which are sensed by VanN. For the LuxS/PQ system, VanS likely synthesizes an AI-2 signal, which is sensed by VanQ (Croxatto et al., 2004; Milton, unpublished data). Signal transmissions from both systems converge onto VanU, leading to a minor repression of VanT expression via VanO. In contrast to other *Vibrio* species, vanT mRNA appears to be stable at low cell population density and is not induced as the cell population density increases suggesting that putative sRNAs do not destabilize vanT mRNA (Croxatto et al., 2004). However, the sRNAs may prevent translation of vanT mRNA by other
mechanisms not yet studied. Unexpected observations suggest that the LuxM/N and LuxS/PQ systems work differently to those of the other vibrios. The phosphotransferase protein, VanU, activates while VanO represses expression of vanT. Both VanN and VanQ repress vanT in the absence of VanU suggesting that these sensors may bypass VanU during signal transmission. VanT regulates its own expression in a complex way by repressing vanT and activating vanOU. Since vanT mRNA amounts do not vary much during growth, these signal systems may be used to limit throughout growth the expression of vanT rather than to induce it. The third system is similar to the V. fischeri LuxI/R system. VanI synthesizes N-(3-oxodecanoyl)-3-homoserine lactone (3-oxo-C10-HSL), which likely binds VanR, a transcriptional activator of VanI (Milton et al., 1997). As seen with V. fischeri, a hierarchical link between the VanM/N and VanS/Q signal systems and the LuxI/R system exists. A null mutation in vanM eliminates the production of all AHLs suggesting that the signal molecules produced by VanM regulate the production of signals via VanI (Milton et al., 2001). The genes regulated by VanR are not yet known. Finally, regulation via quorum sensing in V. anguillarum may be further complicated due to a fourth predicted CqsA/S signal system (Henke and Bassler, 2004a).

Vibrio vulnificus

Pathogenesis

V. vulnificus has three main biotypes. Biotype 1 is associated with human disease, biotype 2 prevails in eel infections, and biotype 3 is found in human vibriosis due to fish handling. Human disease is correlated with contaminated seafood or seawater and is characterized by primary septicaemias, wound infections, and gastrointestinal illnesses. Host susceptibility is a primary factor for disease occurrence. Individuals at risk are those with a suppressed immune defence, high iron levels, and liver disease. The epidemiology and pathogenesis of V. vulnificus has been reviewed recently (Strom and Paranjpye, 2000; Chiang and Chuang, 2003; Gulig et al., 2005). Virulence of V. vulnificus is multifactorial. No one virulence factor has been shown to be the main cause of disease and thus, a definitive role for most is still lacking. An extracellular capsular polysaccharide is strongly correlated to virulence. The capsule protects against serum complement and phagocytosis by macrophages. In addition, an NAD\(^+\)-binding protein involved in K\(^+\) uptake also plays a role in serum resistance. Capsular polysaccharides induce proinflammatory cytokine production in mice and may play a role in septic shock. Lipopolysaccharide of biotype 1 also stimulates proinflammatory cytokine production and activates nitric oxidase synthase, inducing death in rats. Motility may play a role in adherence to host cells. A siderophile, vulnibactin, sequestrers iron from transferrin and lactoferrin and delivers it to the bacterial cell. Extracellular proteins are believed to contribute to the rapid invasiveness and massive tissue destruction during a V. vulnificus infection. However, the roles are still not clearly defined. The 45-kDa metalloprotease VvpE and a 56-kDa cytolysin VvhA cause numerous host cytotoxic responses when injected as pure proteins although mutants carrying null mutations are not decreased in virulence in mice. The cytolysin gene vvhA is expressed during infection of mice supporting a role in virulence. A third toxin, RTX, is likely a major cytotoxin that has a complex interaction with numerous host cell targets though the mechanism is not clear.

Quorum sensing in V. vulnificus

No homologues of AHL synthases are found in V. vulnificus and no AHL signalling molecules have been detected suggesting that neither the V. harveyi-like LuxM/N system nor the V. fischeri LuxI homologue are present (Kim et al., 2003). In addition, V. vulnificus is predicted not to contain the CqsA/S system (Henke and Bassler, 2004a). However, a LuxS homologue with AI-2 autoinducer activity is present (Kim et al., 2003; Gulig et al., 2005). An extracellular capsular polysaccharide is strongly correlated to virulence. The capsule protects against serum complement and phagocytosis by macrophages. In addition, an NAD\(^+\)-binding protein involved in K\(^+\) uptake also plays a role in serum resistance. Capsular polysaccharides induce proinflammatory cytokine production in mice and may play a role in septic shock. Lipopolysaccharide of biotype 1 also stimulates proinflammatory cytokine production and activates nitric oxidase synthase, inducing death in rats. Motility may play a role in adherence to host cells. A siderophile, vulnibactin, sequestrers iron from transferrin and lactoferrin and delivers it to the bacterial cell. Extracellular proteins are believed to contribute to the rapid invasiveness and massive tissue destruction during a V. vulnificus infection. However, the roles are still not clearly defined. The 45-kDa metalloprotease VvpE and a 56-kDa cytolysin VvhA cause numerous host cytotoxic responses when injected as pure proteins although mutants carrying null mutations are not decreased in virulence in mice. The cytolysin gene vvhA is expressed during infection of mice supporting a role in virulence. A third toxin, RTX, is likely a major cytotoxin that has a complex interaction with numerous host cell targets though the mechanism is not clear.
Kawase et al., 2004). V. harveyi homologues to the LuxS/PQ system, LuxU, LuxO, and the LuxR transcriptional regulator, SmcR, are also found (McDougal et al., 2000, 2001; Shao and Hor, 2001; Chen et al., 2003). Moreover, five small RNAs are predicted to be present suggesting that this system works in V. vulnificus as described for V. harveyi (Lenz et al., 2004). Fig. 5 shows the suggested quorum-sensing system for V. vulnificus. LuxS and SmcR positively regulate the metalloprotease VvpE expression and negatively regulate cytolyisin VvhA expression (Shao and Hor, 2001; Kim et al., 2003). Both proteins also stimulate the secretion of the proinflammatory mediators, tumour necrosis factor-alpha and nitric oxide, from murine macrophages challenged with V. vulnificus (Shao and Hor, 2001; Chen et al., 2003). Both proteins also stimulate the secretion of the proinflammatory mediators, tumour necrosis factor-alpha and nitric oxide, from murine macrophages challenged with V. vulnificus suggesting a role in septic shock (Shin et al., 2004). However, LuxS but not SmcR is required for virulence in the iron-overload mouse model. Thus, LuxS may regulate other possible virulence genes independently of the SmcR signalling pathway.

Conclusions

The quorum-sensing mechanisms of vibrios vary in complexity and cellular output even though the components are quite similar. This may reflect the plasticity of the Vibrio genomes to respond to a broad spectrum of activities in a highly variable aquatic environment, including associations with animal tissues. All vibrios analysed so far contain quorum-sensing systems involving LuxO. However, the number of signal transmissions converging on the activator LuxO differs. V. vulnificus is the simplest with only a single circuit so far activating LuxO. V. harveyi and V. cholerae contain three parallel circuits converging on LuxO, two of which are homologous. V. fischeri contains two systems and V. anguillarum contains two identified and a third predicted system that transmits signals through LuxO homologues. In addition, both V. fischeri and V. anguillarum contain a LuxI/R signal system that is linked to the LuxO circuits. These specific signalling systems of vibrios utilize numerous quorum-sensing signals, a phosphotransferase protein (LuxU) that possibly integrates signals external to quorum sensing, and numerous small regulatory RNAs to achieve tighter regulation and/or variability of the cellular response. Why so many signals and such sophistication are required for quorum sensing is not completely clear. However, the V. fischeri studies elegantly show that for this bacterium, the signalling systems aid the sequential allocation of cellular responses as needed for survival, such as initiating and maintaining a symbiotic relationship with the squid host.

Quorum-sensing signalling may have a role in biological diversification. Vibrios encounter numerous ecological challenges, such as UV light, predation, competition, and seasonal variations in seawater including pH, salinity, nutrient levels, and temperature. In general, vibrios associate with animal tissues, which present an additional set of ecological challenges, such as evasion of the immune system, iron limitation, and oxidative stress. Vibrios interact with the animal host either as a pathogen, normal flora, or symbiont and the animal host may be either human, fish, coral, or crustacean. The bacterium may also choose a biofilm or free-living life-style. There are over 50 species of vibrios that share the same general niche. Intraspecies competition is likely sharpened via the multitude of signals produced and variations of the intercellular signalling mechanism of the vibrios. Components of AHL-dependent quorum-sensing systems may evolve rapidly to respond to new signals meeting new demands placed on the bacterium by the environment (Collins et al., 2005). Thus, the acquisition of new niches and new behaviours may be achieved via intercellular signalling suggesting that bacteria could control their activities, environment, and even evolution through intercellular signalling (Manefield et al., 2004). The Vibrio species provide excellent examples of bacteria that have a broad range of highly competitive activities that allow a large number of similar species to coexist in different microniches along with bacteria of other genera.

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