Molecular Microbiology (2012) 84(5), 795–806
doi:10.1111/j.1365-2958.2012.08065.x
First published online 2 May 2012

MicroReview

Shedding light on bioluminescence regulation in *Vibrio fischeri*

Tim Miyashiro* and Edward G. Ruby
Department of Medical Microbiology and Immunology, University of Wisconsin School of Medicine and Public Health, 1550 Linden Drive, Madison, WI 53706-1521, USA.

Summary

The bioluminescence emitted by the marine bacterium *Vibrio fischeri* is a particularly striking result of individual microbial cells co-ordinating a group behaviour. The genes responsible for light production are principally regulated by the LuxR–LuxI quorum-sensing system. In addition to LuxR–LuxI, numerous other genetic elements and environmental conditions control bioluminescence production. Efforts to mathematically model the LuxR–LuxI system are providing insight into the dynamics of this autoinduction behaviour. The Hawaiian squid *Euprymna scolopes* forms a natural symbiosis with *V. fischeri*, and utilizes the symbiont-derived bioluminescence for certain nocturnal behaviours, such as counterillumination. Recent work suggests that the tissue with which *V. fischeri* associates not only can detect bioluminescence but may also use this light to monitor the *V. fischeri* population.

Introduction

For several decades, bacterial bioluminescence has been viewed as the quintessential example of microbial group behaviour. Light production from a single, isolated bacterium appears to be both biologically irrelevant and energetically wasteful. However, the overall emission from a group of cells is sufficiently high that eukaryotic organisms as complex as fish and squid have evolved organs dedicated to housing such bacteria, whose bioluminescence is used during certain nocturnal behaviours, including hunting and counterillumination (Haddock *et al*., 2010). These host–microbe associations, such as the sepiolid squid–vibrio symbiosis, are highly species specific, and have also become model systems for discovering bacterial colonization factors and host specificity determinants (Visick and Ruby, 2006; Mandel, 2010).

The biochemical and genetic mechanisms underlying bacterial bioluminescence are well understood, particularly in members of the *Vibrionaceae* (Meighen, 1993). The genes responsible for light production were first identified in the marine bacterium *Vibrio* (*Aliivibrio*) *fischeri*, which contains two chromosomes, and in some isolates, a large plasmid (Engebrecht *et al*., 1983; Ruby *et al*., 2005). The *luxCDABEG* genes, which are located on the second chromosome, form part of an operon that encodes all of the structural components necessary for light production (Engebrecht *et al*., 1983) (Fig. 1A). At the core of light production is the enzyme luciferase, which is a heterodimer composed of α and β subunits that are encoded by *luxA* and *luxB* respectively. Luciferase releases light during the mixed-function oxidation of a long-chain aldehyde (RCOH) and reduced flavin mononucleotide ($FMNH_2$) (Fig. 1B). LuxD diverts fatty acyl groups from the fatty acid biosynthesis pathway to yield fatty acids for luminescence (Boylan *et al*., 1989). LuxC activates the acyl group with AMP, which is then reduced to the long-chain aldehyde by LuxE. In this manner, LuxC and LuxE are also able to recycle the long-chain fatty acid resulting from the luciferase reaction by reducing it back to its aldehyde form. LuxG was shown to reduce FMN produced by the luciferase reaction (Nijvipakul *et al*., 2008).

The regulation of these bioluminescence genes is inherently linked with quorum sensing, which is the chemical-based form of intercellular communication by which many bacteria co-ordinate population- or community-level behaviours. The term quorum sensing was originally devised to define the general autoinduction phenomenon associated with certain bacterial behaviours, such as the production of bioluminescence by cultures of *V. fischeri* (Fuqua *et al*., 1994). Briefly, as *V. fischeri* cultures grew, a signalling molecule (autoinducer) accumulated in the media. At a certain threshold concentration, the cells would

Accepted 4 April, 2012. *For correspondence. E-mail miyashiro@wisc.edu; Tel. (+1) 608 262 5550; Fax (+1) 608 262 8418.

© 2012 Blackwell Publishing Ltd

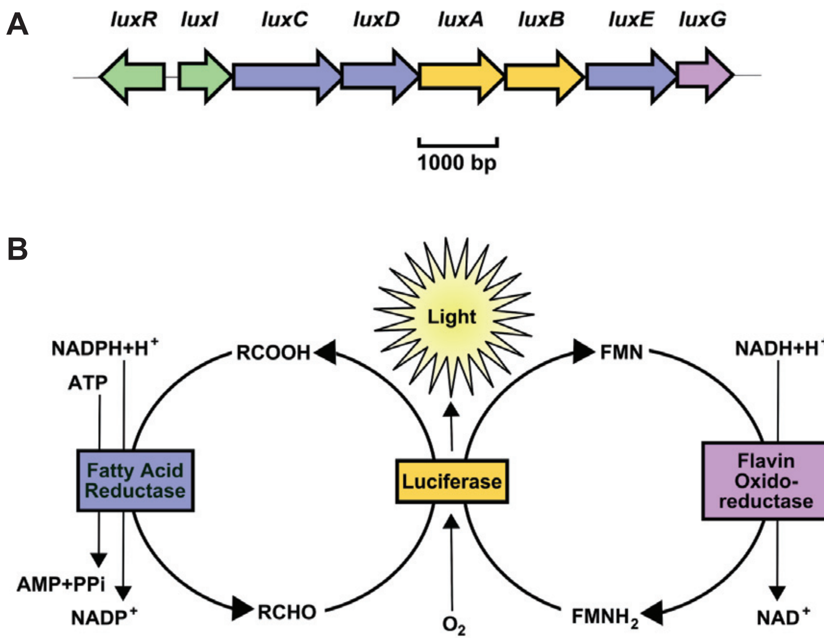

Fig. 1. A. The *lux* locus consists of genes encoding proteins involved in regulation (LuxR and LuxI) and in the production of luminescence (LuxA–G).
B. Enzymatic properties of structural genes within the *lux* operon.

respond to the autoinducer by producing light. The regulatory elements primarily responsible for autoinduction turned out to be LuxI, which synthesizes the autoinducer molecule, and LuxR, an autoinducer-dependent transcription factor (Fig. 2A). The subsequent realization that LuxR–LuxI systems not only are prevalent among bacteria but also regulate genes involved in pathogenesis, biofilm formation, genetic competence and antibiotic production

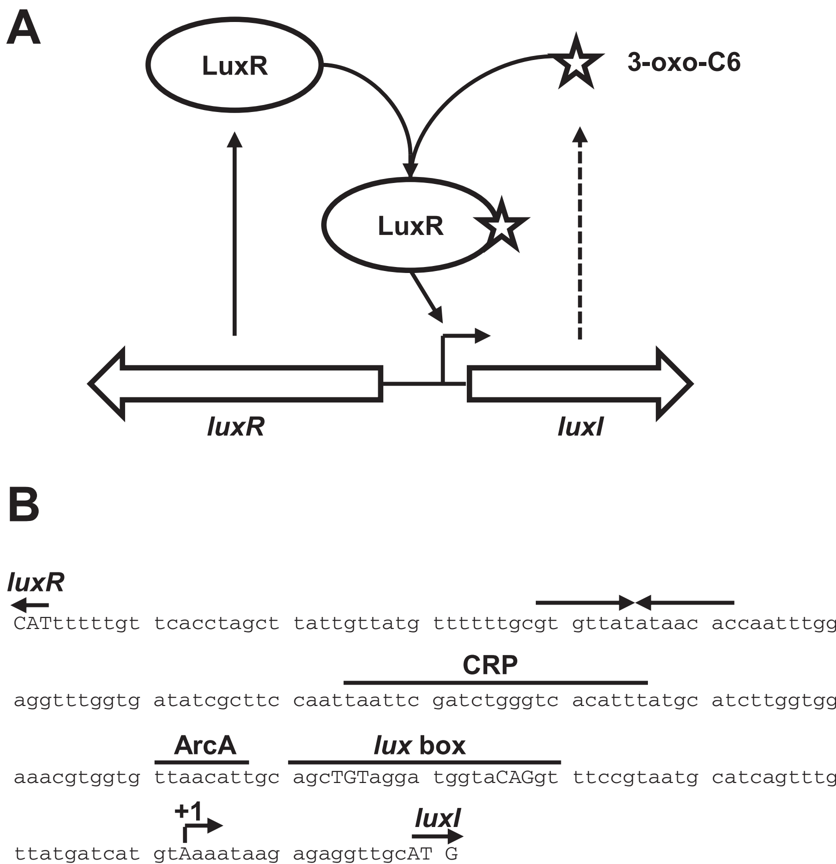

Fig. 2. A. LuxR–LuxI module. The LuxR/3-oxo-C6 complex activates transcription of the *luxI* promoter. Positive feedback at this promoter results in a threshold response to autoinduction.
B. *cis*-regulatory components of the *luxR–luxI* intergenic region. Transcriptional start site of *lux* genes is indicated by the '+1'. Convergent arrows highlight inverted repeat of unknown function. CRP, ArcA and LuxR/3-oxo-C6 (*lux* box) binding sites are shown. Upper case letters within *lux* box have been shown to be important for activation by LuxR/3-oxo-C6.

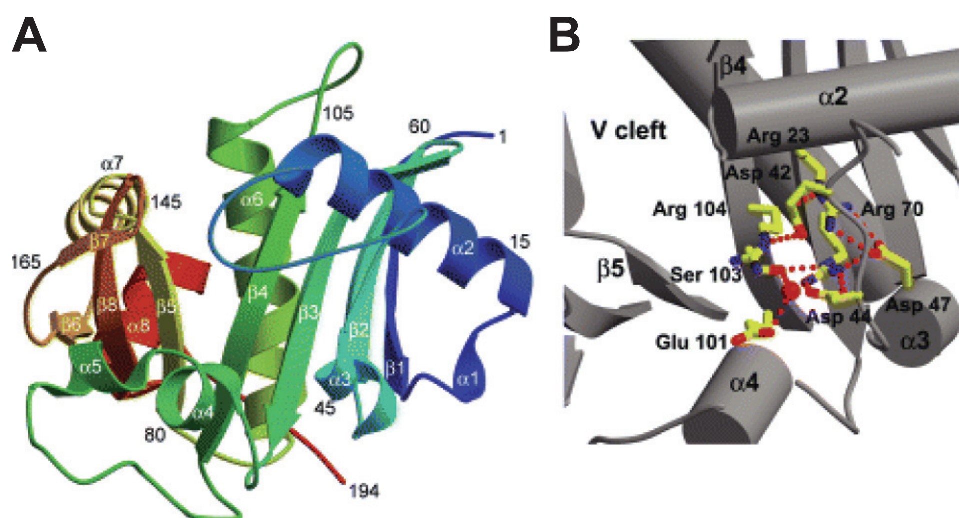

Fig. 3. A. Structure of LasI, the LuxI homologue in *P. aeruginosa*.
B. Electrostatic cluster within LasI.
Images were originally published in Gould *et al*. (2004).

spawned great interest in the field of bacterial quorum sensing.

In this MicroReview, we review what is known about the LuxR–LuxI signalling module in *V. fischeri*. In particular, we focus on the primary components of the LuxR–LuxI module, as well as additional transcription factors that regulate the module *in trans*. In light of the general interests in modelling the dynamics of this module, we also tie together these results to emphasize novel variables to include as modelling parameters.

The core LuxR–LuxI module

LuxI and N*-3-oxohexanoyl-homoserine lactone*

In *V. fischeri*, *luxI* encodes a protein of 193 amino acids that catalyses the synthesis of the autoinducer *N*-3-oxohexanoyl-homoserine lactone (3-oxo-C6) (Schaefer *et al*., 1996). In particular, LuxI converts the substrates *S*-adenosylmethionine (SAM) and hexanoyl-acyl carrier protein (ACP) to three products: 3-oxo-C6, 5′-methylthioadenosine and apo-ACP.

3-oxo-C6 rapidly diffuses across prokaryotic cell membranes and, as a result, the cytoplasmic concentration equilibrates to that present in the surrounding environment (Kaplan and Greenberg, 1985). A relatively low level of 3-oxo-C6 (e.g. 120 nM) is sufficient to induce maximal luminescence output from *V. fischeri* cultures (Lupp *et al*., 2003), highlighting the sensitivity bacteria have evolved towards autoinducer molecules. It is precisely this high level of sensitivity that has encouraged researchers to pursue the production of non-native modulators of quorum-sensing systems in pathogenic microbes (Chen *et al*., 2011; McInnis and Blackwell, 2011).

While the structure of LuxI has not yet been determined, structures of EsaI and LasI, which are LuxI homologues in *Pantoea stewartii* and *Pseudomonas aeruginosa*, respectively, have been solved (Watson *et al*., 2002; Gould *et al*., 2004). Both structures contain a V-shaped cleft formed between beta strands β4 and β5 that forms the active site for acyl transfer from ACP to SAM (Fig. 3A). Next to the V-shaped cleft is a large electrostatic cluster comprised of several residues conserved among LuxI homologues that presumably contributes to protein stability (Fig. 3B). A mutagenesis study of LuxI identified 11 residues critical for the synthesis of autoinducer (Hanzelka *et al*., 1997); six of these residues (R25, E44, D46, D49, R70 and R104) map near or directly to this cluster. Two other residues identified from the screen (A133 and E150) map to alpha helices α6 and α7. Due to the proximity of these alpha helices to the V-shaped cleft, the associated mutations presumably disrupt substrate recognition.

Theoretically, the rate of autoinducer synthesis would have an impact on the timing of autoinduction within a population of cells present in a given volume. One study linked the LuxI catalytic rate of autoinducer production to ampicillin resistance, thereby enabling a selection for LuxI mutants with higher rates of 3-oxo-C6 synthesis (Kambam *et al*., 2008). In particular, an E63G mutation appears to be responsible for an approximately 30-fold increase in 3-oxo-C6 production, although the LuxI levels in these mutants were approximately twofold higher. Interestingly, the LuxI homologues of two symbiotic *V. fischeri* strains, ES114 [isolated from a squid light organ (Boettcher and

Ruby, 1990)] and MJ11 [isolated from a fish light organ (Mandel *et al*., 2009)], produce low and high levels of the 3-oxo-C6 autoinducer, respectively (Stabb *et al*., 2007), and they exhibit three allelic variants in this region (T62A, N64E and S66N for ES114 compared with MJ11), which map to the beta turn located between β2 and β3. Importantly, the relative levels of LuxI within ES114 and MJ11 are unknown, so attributing the different autoinducer levels solely to different catalytic rates between the two alleles is not possible at this time. However, further sequencing and catalytic characterization of LuxI proteins from different *V. fischeri* isolates will reveal the impact of the enzymatic activity of LuxI on bioluminescence production.

LuxR

The *V. fischeri luxR* gene encodes a transcription factor that activates the expression of the *luxICDABEG* operon (Fig. 1A) in response to the presence of 3-oxo-C6 (Fig. 2A). Initial attempts to study LuxR *in vitro* were hampered by the presence of two potential start codons separated by a single codon in the *luxR* gene of *V. fischeri* MJ1, another fish light-organ strain. It was only after N-terminal sequencing of a C-terminal His-tagged LuxR revealed that over 90% of the protein was initiated at the upstream start codon that LuxR was successfully overproduced and isolated in the presence of 3-oxo-C6 (Urbanowski *et al*., 2004). In contrast to strains obtained from fish, only a single possible start codon is present within the *luxR* gene of squid-derived symbionts such as strain ES114. The sequence of *luxR*, as well as the other *lux* genes, exhibits higher diversity than the genes surrounding the locus, consistent with the hypothesis that the *lux* genes are under strong selective pressure that varies in different environments (Bose *et al*., 2011). In fact, serial passaging of bright *V. fischeri* isolates through naïve squid lead to the emergence of descendants with lower luminescence profiles, which are typical of symbionts isolated from wild-caught squid (Schuster *et al*., 2010).

Like LuxI, the structure of LuxR has yet to be determined. However, several mutagenesis studies of LuxR have demonstrated that the autoinducer and DNA-binding domains are distinct. For example, overexpression of the N-terminal two-thirds of LuxR is sufficient to bind autoinducer (Hanzelka and Greenberg, 1995). The remaining C-terminal end of LuxR is sufficient to bind DNA, interact with RNA polymerase, and activate transcription of the *lux* promoter in an autoinducer-independent manner (Choi and Greenberg, 1991; Finney *et al*., 2002). This C-terminal fragment of LuxR also activates the *lux* promoter to higher levels than the full-length protein (Choi and Greenberg, 1991), suggesting that the N-terminal domain prevents LuxR-mediated gene regulation unless bound to autoinducer. The structure of the LuxR-homologue TraR bound to both autoinducer (*N*-3-oxooctanoyl-L-homoserine lactone) and DNA shows that the autoinducer- and DNA-binding domains are separated by a linker (Zhang *et al*., 2002), supporting the interpretations of the results described above. Interestingly, the autoinducer binds irreversibly to TraR, and, based on the structure, is completely encased by the protein (Zhu and Winans, 2001; Zhang *et al*., 2002). In contrast to TraR, LuxR binds 3-oxo-C6 reversibly, a difference that may play a role in the rapid inactivation of *lux* expression that is observed during transition from high to low cell densities (Urbanowski *et al*., 2004). Determining the structure of LuxR/3-oxo-C6 complex will yield mechanistic insight into this reversible binding, which may assist *V. fischeri* to rapidly alter gene regulation by LuxR when cells are suddenly transitioned from high- to low-nutrient environments (e.g. when cells are released from the light organ into seawater).

The LuxR/3-oxo-C6 complex binds to a 20 bp sequence within the *luxR–luxI* intergenic region referred to as the '*lux* box' (Fig. 2B) (Stevens *et al*., 1994). The *lux* box is centred 42.5 bp upstream of the *luxI* promoter start site, indicating the LuxR/3-oxo-C6 complex serves as a transcriptional activator (Egland and Greenberg, 1999). A systematic mutational analysis of the *lux* box demonstrated that base pairs located at positions 3–5 and 16–18 are critical for LuxR regulation of *lux* expression (Antunes *et al*., 2008). The presence of this overall dyad symmetry in the *lux* box supports the general assumption that LuxR regulates gene expression as a dimer. While dimerization of LuxR has yet to be shown, analysis of TraR has shown that this transcription factor acts as a dimer, and that dimerization contributes to the stability of the protein (Pinto and Winans, 2009). Resistance to proteolysis is also conferred by complexing with its cognate autoinducer, *N*-3-oxooctanoyl-L-homoserine lactone, which binds irreversibly to TraR (Zhu and Winans, 2001).

A microarray study has shown that LuxR also regulates genes other than those in the *lux* locus (Antunes *et al*., 2007). Of 25 genes shown to be differentially regulated by at least 2.5-fold in the presence of 2.3 μM 3-oxo-C6 compared with the absence of 3-oxo-C6, 24 genes were upregulated and 1 gene was downregulated. The 25 genes are located within 13 operons, and the LuxR/3-oxo-C6 complex directly binds to 7 of the corresponding promoter elements. Two of the genes identified in this study are of particular interest. *VF_1615*, which encodes a hypothetical protein with an N-terminal transmembrane domain, is the first example of direct negative regulation by LuxR in *V. fischeri*. *VF_A1058*, which is also referred to as *qsrP*, has repeatedly appeared in the literature associated with LuxR regulation (Callahan and Dunlap, 2000), most recently as one of the most highly expressed proteins found in bacteria associated with the squid light organ (Schleicher and Nyholm, 2011). While the function

of QsrP is currently unknown, a *qsrP* mutant displays a defect in colonizing juvenile squid when competed against its parental strain in a co-colonization experiment (Callahan and Dunlap, 2000). Future studies involving *qsrP* mutants of *V. fischeri* will help determine whether QsrP plays a LuxR-mediated role in symbiosis that does not involve luminescence.

Non-luminous strains of V. fischeri

Bioluminescence had been thought to be a defining characteristic of the species *V. fischeri*. However, a recent study reports the first case of non-bioluminescent (i.e. 'dark') strains of *V. fischeri* isolated from the environment (Wollenberg *et al*., 2011). Interestingly, the majority of these dark isolates lack the entire *lux* locus, rather than SNPs or INDELs within specific *lux* genes, as has been reported in other *Vibrio* species (O'Grady and Wimpee, 2008), suggesting that the *lux* locus is lost by natural selection rather than genetic drift. Using *V. fischeri*-specific markers other than the *lux* locus, Wollenberg *et al.* demonstrated that the dark strains examined exhibit polyphyly, i.e. the ancestors of individual isolates that lost the *lux* locus arose independently.

These findings suggest that our knowledge of how *V. fischeri* interacts with the environment has been biased by (i) selection heavily biased towards (visibly) luminescent isolates from natural seawater samples, and (ii) studies of isolates from host environments where bioluminescence is required. What are the selective pressures that result in the loss of bioluminescence? Because the *lux* genes are under control of quorum sensing, planktonic cells within the water column will not express detectable luminescence, suggesting that the evolutionary pressure to lose the *lux* locus exists elsewhere. One possibility is within the sediment, where *V. fischeri* encounters other bacteria that synthesize 3-oxo-C6, and together will achieve a sufficient quorum to induce *lux* gene expression. The general loss of the entire *lux* locus in these strains suggests that it is the bioluminescence genes that are under negative selection, rather than merely LuxR-mediated regulation. However, phylogenetic analyses of other LuxR-regulated genes in these dark strains are necessary to rule out this possibility.

trans regulators of bioluminescence genes

As determined in *V. fischeri* strain MJ1 (Egland and Greenberg, 1999), the regulatory region called the *lux* box is located 42.5 bp upstream of the transcriptional start site of the *luxICDABEG* operon. In addition to LuxR, other regulatory proteins have been shown to regulate expression of the lux locus *in trans* (Fig. 4). A recent analysis comparing the *luxR–luxI* intergenic regions of various other *V. fischeri* isolates revealed high conservation of several base pairs in addition to those shown to be critical for LuxR activity (Bose *et al*., 2011). These bases may be important for DNA conformation and/or recruiting RNA polymerase. This comparative analysis of the *luxR–luxI* intergenic region also revealed that the binding sites of two transcriptional regulators, cAMP receptor protein (CRP) and ArcA (see below), are also relatively conserved. Interestingly, the CRP binding sites displayed somewhat more sequence divergence, which may suggest that the role of catabolite repression may differ due to the particular carbon sources available within the various host environments inhabited by *V. fischeri*.

ArcB–ArcA

The ArcB–ArcA system directly regulates *lux* expression in *V. fischeri* (Bose *et al*., 2007). ArcB is a histidine kinase protein that senses the redox state of the cell's quinone pool. Under conditions that result in reduced quinones [e.g. either oxygen- or reactive-oxygen species (ROS)-poor environments], ArcB will phosphorylate the response regulator ArcA, which regulates expression of a number of genes (Gunsalus and Park, 1994). Two potential ArcA binding sites have been identified in the *luxR–luxI* intergenic region of ES114 (Bose *et al*., 2007); however, only site 1 is conserved among different isolates of *V. fischeri* (Bose *et al*., 2011) (Fig. 2B).

The direct repression of *lux* expression by ArcA in *V. fischeri* is independent of 3-oxo-C6 autoinducer. An intriguing possibility suggested by Bose *et al.* is that the ArcB–ArcA system serves as a feed-forward mechanism to prevent the expression of *lux* genes in the absence of oxygen and to prime bacteria for colonization through the detection of ROS (Bose *et al*., 2007; Small and McFall-Ngai, 1999). Another possibility is that the ArcB–ArcA system enables cells to shut down luminescence as oxygen becomes limiting, even when 3-oxo-C6 levels remain high. Additional experiments comparing the luminescence response to 3-oxo-C6 and oxygen availability will be required to refine these models.

cAMP-CRP

The effect of catabolite repression on luminescence in *V. fischeri* is well documented but poorly understood. Early experiments demonstrated that the presence of glucose in growth medium would repress culture luminescence, and the addition of cAMP would eliminate this repression (Friedrich and Greenberg, 1983). The interpretation of this result is complicated by the observation that the transient addition of cAMP is sufficient to permanently eliminate the repression of luminescence by glucose. The effect on luminescence is thought to be due to activation of *luxR* expression by the direct binding of the CRP/cAMP complex to a specific site within the *luxR–luxI* intergenic region (Fig. 2B). CRP/cAMP binding to the *luxR–luxI* intergenic region also represses *lux* transcription; however, levels of LuxR above a threshold value are sufficient to overcome this repression during autoinduction.

Very little research examining the regulatory role of CRP/cAMP on luminescence in *V. fischeri* has been conducted in the past two decades. However, recent examination of the transcriptome of natural *V. fischeri*

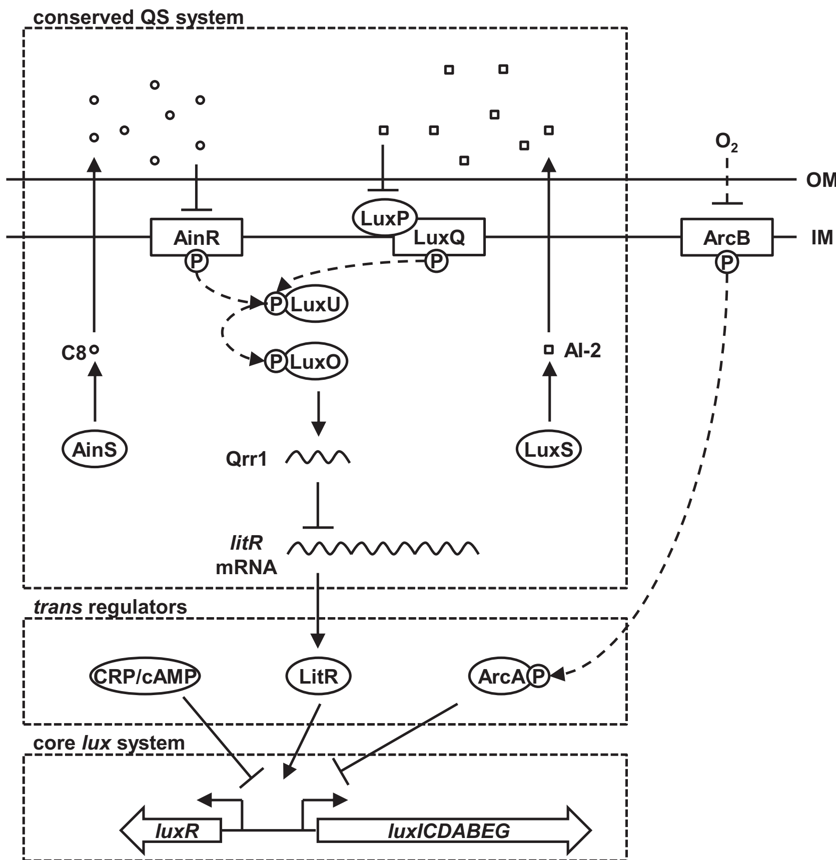

Fig. 4. Multiple systems regulate expression of the *lux* locus in *V. fischeri*. The relative kinase/phosphatase activities of the histidine kinases AinR and LuxQ towards the HPT protein LuxU are determined by the concentrations of C8 and AI-2 respectively. Phosphorylation of the response regulator LuxO by LuxU leads to expression of *qrr1*, which encodes a sRNA that post-transcriptionally represses *litR* transcript. LitR directly regulates the expression of *luxR*. Also shown are CRP/cAMP and ArcA-P, which directly regulate the expression of the *lux* genes.

populations within adult squid light organs has revealed complex metabolic patterns that vary according to the diel rhythm (Wier *et al*., 2010). In particular, genes involved in the utilization of *N*-acetylglucosamine (GlcNAc) and its polymeric form, chitin, are elevated at night, when the squid uses the symbiont's bioluminescence. The repressor NagC regulates a subset of these genes as well as other GlcNAc-utilization genes such as *nagE*, *nagA* and *nagB* (Miyashiro *et al*., 2011). While the effect of catabolite repression on these genes is unknown in *V. fischeri*, CRP has been shown to bind upstream of their homologues in *Escherichia coli* (Plumbridge and Kolb, 1991). In light of the sequence diversity exhibited by different *V. fischeri* isolates at the CRP binding site within the *luxR–luxI* intergenic region (Bose *et al*., 2011), it would be interesting to determine whether the levels of luminescence produced by these isolates also show a rhythm within the squid.

LitR

The transcriptional regulator LitR was first identified through a search for the *V. fischeri* homologue of LuxR in *Vibrio harveyi* (no relation to the *V. fischeri* LuxR-like protein family), and HapR in *Vibrio cholerae* (Fidopiastis *et al*., 2002). LitR was shown to directly bind a fragment of DNA upstream of the *V. fischeri luxR* gene, independent of the *lux* box, and enhance *luxR* expression without affecting expression of the other *lux* genes, as measured by transcription of *luxC*.

Subsequent studies have revealed that LitR is indeed a pleiotropic regulator. For instance, LitR negatively regulates both soft-agar motility (Lupp and Ruby, 2005) and colonization of the squid light organ (Fidopiastis *et al*., 2002; Miyashiro *et al*., 2010). In addition to *luxR* expression, LitR positively regulates expression of *acs* and *rpoQ*, which in turn regulate two symbiosis-related activities: organic-acid secretion and chitinase activity respectively (Cao *et al*., 2012; Lupp and Ruby, 2005; Studer *et al*., 2008). Further studies are required to determine the complete LitR regulon, which may yield insight into the factors within the regulon of HapR that enable *V. cholerae* to colonize the human gut (Tsou *et al*., 2009).

Other QS systems in *V. fischeri*

AinS–AinR and LuxS–LuxP/Q

Vibrio fischeri regulates luminescence with at least two other quorum-sensing systems, which are based on the autoinducer molecules *N*-octanoyl-homoserine lactone (C8) and autoinducer-2 (AI-2), a furanosyl borate diester

(Fig. 4). C8 is synthesized by AinS, and detected by the histidine kinase AinR and LuxR (Lupp *et al*., 2003). As in all *Vibrio* spp., AI-2 is synthesized by LuxS and detected by LuxP, a periplasmic protein that interacts with the histidine-kinase sensor LuxQ (Neiditch *et al*., 2006).

The AinS–AinR system has garnered considerable attention in the *V. fischeri* field because it exerts a greater impact on bioluminescence and host colonization than the parallel LuxS–LuxP/Q system (Lupp and Ruby, 2004). Specifically, the luminescence emitted by an *ainS* mutant, in broth culture or within the squid light organ, is attenuated compared with wild-type cells. Interestingly, the AinS–AinR system appears to be specific to the *Aliivibrio* clade of *Vibrionaceae* (which includes *V. fischeri*), although weak homology does exist with the LuxM–LuxN system found in *V. harveyi*; however, LuxM synthesizes a structurally distinct hydroxyl autoinducer, *N*-(3-hydroxybutanoyl) homoserine lactone. In the absence of C8, AinR is thought to act as a kinase towards the histidine phosphotransferase (HPT) protein LuxU. Direct biochemical evidence of this interaction has yet to be reported; nevertheless, such phosphoryl-based interactions between LuxN and LuxU have been demonstrated in *V. harveyi* (Timmen *et al*., 2006).

LuxP/Q, which is conserved in the *Vibrionaceae*, also can phosphorylate LuxU when the AI-2 concentration is low. However, signalling by AI-2 appears to be inconsequential during the initiation of symbiosis by *V. fischeri*, because a Δ*luxS* mutant displays wild-type levels of bioluminescence and host colonization (Lupp and Ruby, 2004). Only in the absence of C8 signalling does the presence of AI-2 have a detectable effect on these activities.

The role of small RNAs in QS

The LuxO module, which consists of LuxU, LuxO and the small regulatory RNA Qrr1, are genetically linked and conserved among the *Vibrionaceae*. In *V. fischeri*, the *luxOU* and *qrr1* operons are transcribed from divergent promoters, based on similar genetic architectures in *V. harveyi* and *V. cholerae* (Lenz *et al*., 2004; Miyashiro *et al*., 2010; Tu *et al*., 2010).

The HPT protein LuxU serves as an intermediate component within the phosphorelay. The convergence of signals from at least two histidine kinases (AinR and LuxQ) onto a single protein (LuxU) is an example of a ‘many-to-one’ branched pathway prevalent among bacterial two-component signalling systems (Laub and Goulian, 2007). This branched relationship involving LuxU is also a shared trait in the *Vibrionaceae* (Milton, 2006), and the apparent promiscuity of LuxU may, in fact, enable different histidine-kinase sensors to access the LuxO module more easily through evolutionary convergence or divergence. Investigation into the specificity determinants enabling the conserved, branched network involving LuxU will yield insight into a regulatory pathway that is fundamental to quorum sensing in this bacterial family.

LuxO is a σ^{54}-dependent response regulator that transcriptionally activates *qrr1* when phosphorylated (Lilley and Bassler, 2000; Miyashiro *et al*., 2010). Characterization of LuxO binding sites in *V. harveyi* suggests that the response regulator recognizes the consensus sequence ‘TTGCAWWWTGCAA’ found upstream of each of the five *qrr* genes in this species (Tu and Bassler, 2007; Tu *et al*., 2010). In *V. fischeri*, there are only two putative binding sites found in the entire genome, both of which are located in the *qrr1–luxOU* intergenic region, and are separated by 21 bp. Determining how these two binding sites impact the regulation of *qrr1*, and possibly *luxOU*, requires further investigation, and may yield interesting regulatory patterns associated with the level of phosphorylated LuxO.

The Qrr class of small regulatory RNAs was originally discovered during a genetic screen that had identified the RNA chaperone Hfq as a negative regulator of bioluminescence in *V. harveyi* (Lenz *et al*., 2004). Further characterization revealed that the *V. harveyi* and *V. cholerae* genomes contain five and four *qrr* homologues, respectively, that each have the capacity to post-transcriptionally control the transcript level of their LitR master regulator homologues (e.g. LuxR and HapR). Direct base-pairing between *hapR* mRNA and a 21 bp region conserved among Qrrs has recently been shown in *V. cholerae* (Bardill *et al*., 2011). Several other vibrio mRNAs are also controlled post-transcriptionally by direct Qrr association with the same region of the sRNAs (Hammer and Bassler, 2007; Rutherford *et al*., 2011; Svenningsen *et al*., 2009). Subsequent work has demonstrated that individual Qrrs may differentially regulate other targets, due to additional pairing that includes sRNA nucleotides that are not conserved but specific to a single Qrr (Shao and Bassler, 2012). However, a unifying model to explain the general role of multiple *qrr* genes has remained elusive.

We recently examined the evolutionary history of the LuxO module by performing phylogenetic analyses of the *luxO* and *litR* homologues from all fully sequenced *Vibrionaceae* members (Miyashiro *et al*., 2010). Resulting phylograms were found to have congruent topology, suggesting that these two signalling proteins have had a similar evolutionary history during the divergence of the *Vibrionaceae*. Remarkably, the number of *qrr* genes found within the genome of each *Vibrionaceae* member clustered according to its position on the phylogenetic tree. In particular, the species within the *Aliivibrio* clade, which includes *V. fischeri*, possess only a single *qrr* gene (*qrr1*), which is linked to its *luxOU* operon. One parsimonious hypothesis is that the single *qrr* gene found in *V. fischeri* is most closely related to an ancestral qrr gene from which

the multiple other qrr paralogues have derived (Tu and Bassler, 2007; Tu *et al*., 2010). A functional examination of *qrr1* in *V. fischeri* revealed that Qrr1 is both necessary and sufficient for LuxO-mediated regulation of LitR, further supporting its ancestral position (Miyashiro *et al*., 2010). Discovering the biological advantage that drove the multiplication and diversification of *qrr* paralogues in some clades of *Vibrionaceae* will likely be challenging but will ultimately contribute to our understanding of the evolution of quorum signalling in these and other bacteria.

Host responses to V. fischeri *QS*

The squid–vibrio symbiosis has offered a unique system to examine the role of bioluminescence, as well as bacterial quorum sensing, in a natural host environment (McFall-Ngai *et al*., 2011). Juvenile squid colonized by strains of *V. fischeri* containing mutations in *luxA*, *luxI* or *luxR* do not emit detectable levels of luminescence (Visick *et al*., 2000). Even in this early study, there was evidence that the host can sanction dark strains of *V. fischeri*, as their corresponding colonization levels have dropped significantly by 48 h post inoculation. Furthermore, when colonized by these dark mutants, the epithelial cells of the light organ failed to swell, which is a normal developmental step during establishment of the symbiosis (Montgomery and McFall-Ngai, 1994).

More recently, a microarray-based study compared the host transcriptome of uncolonized light organs with those colonized by wild type, Δ*luxI* or Δ*luxA V. fischeri* strains (Chun *et al*., 2008). The presence of the bacterial symbionts (wild type, Δ*luxI* or Δ*luxA*) was sufficient to activate transcription of immune-related components, such as LPS-binding protein and PGN-recognition proteins, as well as of visual transduction cascade proteins, such as guanylate cyclase. The upregulation of these genes in light organs colonized by Δ*luxI* or Δ*luxA* mutants suggests it is the initial contact between the host and potential symbiont, rather than quorum sensing or luminescence production, which activates the developmental pathways that prepare the light organ to maintain a light-producing bacterial population. Consistent with the theory that the host can monitor the luminescence output of the light organ, recent electroretinogram studies have demonstrated that the light organ, which contains visual transduction cascade proteins like opsin, arrestin and rhodopsin kinase, does physiologically respond to light cues (Tong *et al*., 2009; McFall-Ngai *et al*., 2011). Whether signalling outputs of the visual transduction cascade enable the host to specifically sanction (i.e. eliminate) non-luminous *V. fischeri* cells awaits further studies.

One luminescence-dependent effect observed in the microarray-based study described above was higher transcriptional activity of the gene encoding haemocyanin, which is a cell-free, oxygen-binding protein analogous to haemoglobin (Chun *et al*., 2008). An intriguing possibility is that the host uses haemocyanin to provide oxygen to the light-organ crypts for the enzymatic activity of luciferase (N. Kremer and M. McFall-Ngai, pers. comm.). Continued focus on the enzymatic substrates and products of luciferase within the context of the squid–vibrio symbiosis will certainly provide insight into the evolution of host–microbe interactions.

Network analysis of the LuxR–LuxI module

The semi-autonomous nature of the LuxR–LuxI module enabled the initial analyses of autoinduction dynamics to be performed in *E. coli* (Engebrecht *et al*., 1983). These studies led to a long-standing research focus on determining the role of positive feedback within the LuxR–LuxI system, which arises from the positive regulation of the 3-oxo-C6 synthase-encoding *luxI* gene by the LuxR/3-oxo-C6 complex (Fig. 2A). In general, positive feedback within regulatory circuits is often associated with bistability, which describes the presence of two steady states (i.e. on and off) over a range of stimuli. However, the mere presence of positive feedback is not sufficient to guarantee such behaviour. In fact, analysis of the LuxR–LuxI system demonstrated that the pattern of luminescence production by *V. fischeri* as a function of cell density was consistent with a threshold response, which allows only one stable state for each stimulus level (Haseltine and Arnold, 2008). However, using the same core components (LuxR, LuxI, and positive feedback), the authors were able to design regulatory-circuit architectures that yielded graded, threshold or bistable responses to cell density. This study serves as an important reminder that the overall response of a regulatory network depends critically both on the architecture and on the properties of the individual components. Interestingly, another study using different LuxR–LuxI constructs reported bistability in *lux* expression due to positive feedback on *luxR* expression during autoinduction (Williams *et al*., 2008). The discrepancies in the results between the two studies described above highlight a potential limitation in studying the LuxR–LuxI module outside of its natural context. Ultimately, the individual needs of each system will drive the evolution of a regulatory pattern that provides the optimal function within the biological context of each bacterial species (Milton, 2006).

A recent study of the *V. fischeri* LuxR–LuxI system has revealed that individual cells within a population can respond differently to a homogeneous concentration of autoinducer (Perez and Hagen, 2010). An important characteristic of this work was the use of *V. fischeri* cells, rather than LuxR–LuxI plasmid constructs in *E. coli*, as the basis for the analyses. Performing the analyses within the system's native genetic background assures that any pattern of quorum-signalling activity is the sum of the multiple, *V. fischeri*-specific, regulatory inputs discussed above. While the average luminescence output of a population of *V. fischeri* cells in response to autoinducer matches that of bulk culture, the associated variation among the individuals remained large, even at saturating levels of the autoinducer 3-oxo-C6. Although the biological impact of cell-to-cell variation in luminescence is unclear, this population heterogeneity was also observed at the level of *luxI* expression, as measured by fluorescent reporter constructs. Interestingly, heterogeneity in luminescence production has also been observed among a population of *V. harveyi* cells, which lack the LuxR/LuxI module (Anetzberger *et al*., 2009). Such heterogeneity may arise from the generation of transcriptional noise by

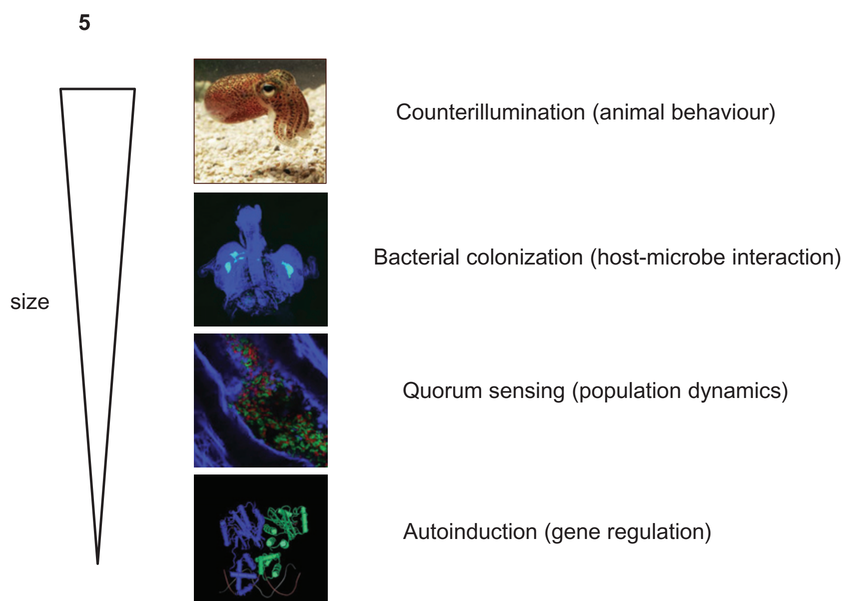

Fig. 5. Areas of research based on *V. fischeri* bioluminescence. Shown in descending order are (i) *E. scolopes* casting a shadow that is eliminated by counterillumination at night, (ii) light organ colonized by *V. fischeri*, (iii) *V. fischeri* cell–cell interactions within the light organ crypts, and (iv) LuxR–LuxI regulation of *lux* genes (shown is a TraR/autoinducer dimer complex bound to DNA).

the multiple feedback loops present within the *V. harveyi* quorum-sensing network (Teng *et al.*, 2011). Whether such cell–cell heterogeneity in *lux* gene expression or luminescence output is relevant in general within natural environments (e.g. squid light organ) requires further study.

There has also been recent interest in examining the impact of multiple types of autoinducer molecules, particularly the AinS-derived autoinducer C8, on the expression of the *lux* genes in *V. fischeri*. One study provided a detailed mathematical model that captures the dual functions of C8, namely as a positive regulator of luminescence by interacting with either AinR or LuxR (Kuttler and Hense, 2008). By adjusting the relative binding affinities between LuxR and either C8 or 3-oxo-C6, the model can account for the different luminescence profiles exhibited by ES114 or MJ1. A different study demonstrated that individual cells display heterogeneous responses in *lux* expression levels regardless of the combined levels of C8 and 3-oxo-C6 (Perez *et al.*, 2011). Together these studies highlight the complex regulatory network that *V. fischeri* uses to control luminescence production.

Conclusions and future directions

Bacterial bioluminescence continues to appeal to the curiosity of the scientific community due to its role in different biological contexts (Fig. 5). The genetic tractability of *V. fischeri*, along with its symbiotic relationship with animal hosts like *Euprymna scolopes*, has enabled this bacterium to significantly contribute to our understanding of fundamental microbiological themes, such as sociomicrobiology (Parsek and Greenberg, 2005) and host–microbe interactions (Ruby, 2008; McFall-Ngai *et al.*, 2011). As genetic and environmental factors controlling *lux* expression are identified, their overall contributions to bioluminescence must be examined and potentially refined within the larger regulatory network, thereby making mathematical modelling an important window through which to study *V. fischeri* bioluminescence.

Acknowledgements

We thank B. Hammer, M. McFall-Ngai and M. Wollenberg for their careful review of the manuscript. We also thank M. Wollenberg for providing Fig. 1. We thank the two anonymous reviewers for their constructive criticisms of the manuscript. This work was supported by NIH Grant RR12294 to E.G.R. and M. McFall-Ngai, by NSF Grant IOS-0817232 to M. McFall-Ngai and E.G.R., and by award 1K99GM097032 from the NIGMS to T.M.

References

Anetzberger, C., Pirch, T., and Jung, K. (2009) Heterogeneity in quorum sensing-regulated bioluminescence of *Vibrio harveyi*. *Mol Microbiol* **73:** 267–277.

Antunes, L.C., Schaefer, A.L., Ferreira, R.B., Qin, N., Stevens, A.M., Ruby, E.G., and Greenberg, E.P. (2007) Transcriptome analysis of the *Vibrio fischeri* LuxR–LuxI regulon. *J Bacteriol* **189:** 8387–8391.

Antunes, L.C., Ferreira, R.B., Lostroh, C.P., and Greenberg, E.P. (2008) A mutational analysis defines *Vibrio fischeri* LuxR binding sites. *J Bacteriol* **190:** 4392–4397.

Bardill, J.P., Zhao, X., and Hammer, B.K. (2011) The *Vibrio cholerae* quorum sensing response is mediated by Hfq-dependent sRNA/mRNA base pairing interactions. *Mol Microbiol* **80:** 1381–1394.

Boettcher, K.J., and Ruby, E.G. (1990) Depressed light emission by symbiotic *Vibrio fischeri* of the sepiolid squid *Euprymna scolopes*. *J Bacteriol* **172:** 3701–3706.
Bose, J.L., Kim, U., Bartkowski, W., Gunsalus, R.P., Overley, A.M., Lyell, N.L., *et al*. (2007) Bioluminescence in *Vibrio fischeri* is controlled by the redox-responsive regulator ArcA. *Mol Microbiol* **65:** 538–553.
Bose, J.L., Wollenberg, M.S., Colton, D.M., Mandel, M.J., Septer, A.N., Dunn, A.K., and Stabb, E.V. (2011) Contribution of rapid evolution of the *luxR–luxI* intergenic region to the diverse bioluminescence outputs of *Vibrio fischeri* strains isolated from different environments. *Appl Environ Microbiol* **77:** 2445–2457.
Boylan, M., Miyamoto, C., Wall, L., Graham, A., and Meighen, E. (1989) Lux C, D and E genes of the *Vibrio fischeri* luminescence operon code for the reductase, transferase, and synthetase enzymes involved in aldehyde biosynthesis. *Photochem Photobiol* **49:** 681–688.
Callahan, S.M., and Dunlap, P.V. (2000) LuxR- and acyl-homoserine-lactone-controlled non-*lux* genes define a quorum-sensing regulon in *Vibrio fischeri*. *J Bacteriol* **182:** 2811–2822.
Cao, X., Studer, S.V., Wassarman, K., Zhang, Y., Ruby, E.G., and Miyashiro, T. (2012) The novel sigma factor-like regulator RpoQ controls luminescence, chitinase activity, and motility in *Vibrio fischeri*. *MBio* **3:** e00285-11.
Chen, G., Swem, L.R., Swem, D.L., Stauff, D.L., O'Loughlin, C.T., Jeffrey, P.D., *et al*. (2011) A strategy for antagonizing quorum sensing. *Mol Cell* **42:** 199–209.
Choi, S.H., and Greenberg, E.P. (1991) The C-terminal region of the *Vibrio fischeri* LuxR protein contains an inducer-independent lux gene activating domain. *Proc Natl Acad Sci USA* **88:** 11115–11119.
Chun, C.K., Troll, J.V., Koroleva, I., Brown, B., Manzella, L., Snir, E., *et al*. (2008) Effects of colonization, luminescence, and autoinducer on host transcription during development of the squid–vibrio association. *Proc Natl Acad Sci USA* **105:** 11323–11328.
Egland, K.A., and Greenberg, E.P. (1999) Quorum sensing in *Vibrio fischeri*: elements of the *luxl* promoter. *Mol Microbiol* **31:** 1197–1204.
Engebrecht, J., Nealson, K., and Silverman, M. (1983) Bacterial bioluminescence: isolation and genetic analysis of functions from *Vibrio fischeri*. *Cell* **32:** 773–781.
Fidopiastis, P.M., Miyamoto, C.M., Jobling, M.G., Meighen, E.A., and Ruby, E.G. (2002) LitR, a new transcriptional activator in *Vibrio fischeri*, regulates luminescence and symbiotic light organ colonization. *Mol Microbiol* **45:** 131–143.
Finney, A.H., Blick, R.J., Murakami, K., Ishihama, A., and Stevens, A.M. (2002) Role of the C-terminal domain of the alpha subunit of RNA polymerase in LuxR-dependent transcriptional activation of the *lux* operon during quorum sensing. *J Bacteriol* **184:** 4520–4528.
Friedrich, W.F., and Greenberg, E.P. (1983) Glucose repression of luminescence and luciferase in *Vibrio-fischeri*. *Arch Microbiol* **134:** 87–91.
Fuqua, W.C., Winans, S.C., and Greenberg, E.P. (1994) Quorum sensing in bacteria: the LuxR–LuxI family of cell density-responsive transcriptional regulators. *J Bacteriol* **176:** 269–275.
Gould, T.A., Schweizer, H.P., and Churchill, M.E. (2004) Structure of the *Pseudomonas aeruginosa* acyl-homoserinelactone synthase LasI. *Mol Microbiol* **53:** 1135–1146.
Gunsalus, R.P., and Park, S.J. (1994) Aerobic-anaerobic gene regulation in *Escherichia coli*: control by the ArcAB and Fnr regulons. *Res Microbiol* **145:** 437–450.
Haddock, S.H., Moline, M.A., and Case, J.F. (2010) Bioluminescence in the sea. *Annu Rev Mar Sci* **2:** 443–493.
Hammer, B.K., and Bassler, B.L. (2007) Regulatory small RNAs circumvent the conventional quorum sensing pathway in pandemic *Vibrio cholerae*. *Proc Natl Acad Sci USA* **104:** 11145–11149.
Hanzelka, B.L., and Greenberg, E.P. (1995) Evidence that the N-terminal region of the *Vibrio fischeri* LuxR protein constitutes an autoinducer-binding domain. *J Bacteriol* **177:** 815–817.
Hanzelka, B.L., Stevens, A.M., Parsek, M.R., Crone, T.J., and Greenberg, E.P. (1997) Mutational analysis of the *Vibrio fischeri* LuxI polypeptide: critical regions of an autoinducer synthase. *J Bacteriol* **179:** 4882–4887.
Haseltine, E.L., and Arnold, F.H. (2008) Implications of rewiring bacterial quorum sensing. *Appl Environ Microbiol* **74:** 437–445.
Kambam, P.K., Sayut, D.J., Niu, Y., Eriksen, D.T., and Sun, L. (2008) Directed evolution of LuxI for enhanced OHHL production. *Biotechnol Bioeng* **101:** 263–272.
Kaplan, H.B., and Greenberg, E.P. (1985) Diffusion of autoinducer is involved in regulation of the *Vibrio fischeri* luminescence system. *J Bacteriol* **163:** 1210–1214.
Kuttler, C., and Hense, B.A. (2008) Interplay of two quorum sensing regulation systems of *Vibrio fischeri*. *J Theor Biol* **251:** 167–180.
Laub, M.T., and Goulian, M. (2007) Specificity in two-component signal transduction pathways. *Annu Rev Genet* **41:** 121–145.
Lenz, D.H., Mok, K.C., Lilley, B.N., Kulkarni, R.V., Wingreen, N.S., and Bassler, B.L. (2004) The small RNA chaperone Hfq and multiple small RNAs control quorum sensing in *Vibrio harveyi* and *Vibrio cholerae*. *Cell* **118:** 69–82.
Lilley, B.N., and Bassler, B.L. (2000) Regulation of quorum sensing in *Vibrio harveyi* by LuxO and sigma-54. *Mol Microbiol* **36:** 940–954.
Lupp, C., and Ruby, E.G. (2004) *Vibrio fischeri* LuxS and AinS: comparative study of two signal synthases. *J Bacteriol* **186:** 3873–3881.
Lupp, C., and Ruby, E.G. (2005) *Vibrio fischeri* uses two quorum-sensing systems for the regulation of early and late colonization factors. *J Bacteriol* **187:** 3620–3629.
Lupp, C., Urbanowski, M., Greenberg, E.P., and Ruby, E.G. (2003) The *Vibrio fischeri* quorum-sensing systems *ain* and *lux* sequentially induce luminescence gene expression and are important for persistence in the squid host. *Mol Microbiol* **50:** 319–331.
McFall-Ngai, M., Heath-Heckman, E.A., Gillette, A.A., Peyer, S.M., and Harvie, E.A. (2011) The secret languages of coevolved symbioses: insights from the *Euprymna scolopes–Vibrio fischeri* symbiosis. *Semin Immunol* **24:** 3–8.
McInnis, C.E., and Blackwell, H.E. (2011) Thiolactone modulators of quorum sensing revealed through library design and screening. *Bioorg Med Chem* **19:** 4820–4828.

Mandel, M.J. (2010) Models and approaches to dissect host-symbiont specificity. *Trends Microbiol* **18:** 504–511.

Mandel, M.J., Wollenberg, M.S., Stabb, E.V., Visick, K.L., and Ruby, E.G. (2009) A single regulatory gene is sufficient to alter bacterial host range. *Nature* **458:** 215–218.

Meighen, E.A. (1993) Bacterial bioluminescence: organization, regulation, and application of the *lux* genes. *FASEB J* **7:** 1016–1022.

Milton, D.L. (2006) Quorum sensing in vibrios: complexity for diversification. *Int J Med Microbiol* **296:** 61–71.

Miyashiro, T., Wollenberg, M.S., Cao, X., Oehlert, D., and Ruby, E.G. (2010) A single *qrr* gene is necessary and sufficient for LuxO-mediated regulation in *Vibrio fischeri*. *Mol Microbiol* **77:** 1556–1567.

Miyashiro, T., Klein, W., Oehlert, D., Cao, X., Schwartzman, J., and Ruby, E.G. (2011) The *N*-acetyl-D-glucosamine repressor NagC of *Vibrio fischeri* facilitates colonization of *Euprymna scolopes*. *Mol Microbiol* **82:** 894–903.

Montgomery, M.K., and McFall-Ngai, M. (1994) Bacterial symbionts induce host organ morphogenesis during early postembryonic development of the squid *Euprymna scolopes*. *Development* **120:** 1719–1729.

Neiditch, M.B., Federle, M.J., Pompeani, A.J., Kelly, R.C., Swem, D.L., Jeffrey, P.D., *et al*. (2006) Ligand-induced asymmetry in histidine sensor kinase complex regulates quorum sensing. *Cell* **126:** 1095–1108.

Nijvipakul, S., Wongratana, J., Suadee, C., Entsch, B., Ballou, D.P., and Chaiyen, P. (2008) LuxG is a functioning flavin reductase for bacterial luminescence. *J Bacteriol* **190:** 1531–1538.

O'Grady, E.A., and Wimpee, C.F. (2008) Mutations in the *lux* operon of natural dark mutants in the genus *Vibrio*. *Appl Environ Microbiol* **74:** 61–66.

Parsek, M.R., and Greenberg, E.P. (2005) Sociomicrobiology: the connections between quorum sensing and biofilms. *Trends Microbiol* **13:** 27–33.

Perez, P.D., and Hagen, S.J. (2010) Heterogeneous response to a quorum-sensing signal in the luminescence of individual *Vibrio fischeri*. *PLoS ONE* **5:** e15473.

Perez, P.D., Weiss, J.T., and Hagen, S.J. (2011) Noise and crosstalk in two quorum-sensing inputs of *Vibrio fischeri*. *BMC Syst Biol* **5:** 153.

Pinto, U.M., and Winans, S.C. (2009) Dimerization of the quorum-sensing transcription factor TraR enhances resistance to cytoplasmic proteolysis. *Mol Microbiol* **73:** 32–42.

Plumbridge, J., and Kolb, A. (1991) CAP and Nag repressor binding to the regulatory regions of the *nagE-B* and *manX* genes of *Escherichia coli*. *J Mol Biol* **217:** 661–679.

Ruby, E.G. (2008) Symbiotic conversations are revealed under genetic interrogation. *Nat Rev Microbiol* **6:** 752–762.

Ruby, E.G., Urbanowski, M., Campbell, J., Dunn, A., Faini, M., Gunsalus, R., *et al*. (2005) Complete genome sequence of *Vibrio fischeri*: a symbiotic bacterium with pathogenic congeners. *Proc Natl Acad Sci USA* **102:** 3004–3009.

Rutherford, S.T., van Kessel, J.C., Shao, Y., and Bassler, B.L. (2011) AphA and LuxR/HapR reciprocally control quorum sensing in vibrios. *Genes Dev* **25:** 397–408.

Schaefer, A.L., Val, D.L., Hanzelka, B.L., Cronan, J.E., Jr, and Greenberg, E.P. (1996) Generation of cell-to-cell signals in quorum sensing: acyl homoserine lactone synthase activity of a purified *Vibrio fischeri* LuxI protein. *Proc Natl Acad Sci USA* **93:** 9505–9509.

Schleicher, T.R., and Nyholm, S.V. (2011) Characterizing the host and symbiont proteomes in the association between the Bobtail squid, *Euprymna scolopes*, and the bacterium, *Vibrio fischeri*. *PLoS ONE* **6:** e25649.

Schuster, B.M., Perry, L.A., Cooper, V.S., and Whistler, C.A. (2010) Breaking the language barrier: experimental evolution of non-native *Vibrio fischeri* in squid tailors luminescence to the host. *Symbiosis* **51:** 85–96.

Shao, Y., and Bassler, B.L. (2012) Quorum-sensing non-coding small RNAs use unique pairing regions to differentially control mRNA targets. *Mol Microbiol* **83:** 599–611.

Small, A.L., and McFall-Ngai, M.J. (1999) Halide peroxidase in tissues that interact with bacteria in the host squid *Euprymna scolopes*. *J Cell Biochem* **72:** 445–457.

Stabb, E.V., Schaefer, A.L., Bose, J., and Ruby, E.G. (2007) Quorum signaling and symbiosis in the marine luminous bacterium *Vibrio fischeri*. In *Cell–Cell Signaling in Bacteria*. Winans, S.C., and Bassler, B. (eds). Washington, DC: ASM Press, pp. 233–250.

Stevens, A.M., Dolan, K.M., and Greenberg, E.P. (1994) Synergistic binding of the *Vibrio fischeri* LuxR transcriptional activator domain and RNA polymerase to the *lux* promoter region. *Proc Natl Acad Sci USA* **91:** 12619–12623.

Studer, S.V., Mandel, M.J., and Ruby, E.G. (2008) AinS quorum sensing regulates the *Vibrio fischeri* acetate switch. *J Bacteriol* **190:** 5915–5923.

Svenningsen, S.L., Tu, K.C., and Bassler, B.L. (2009) Gene dosage compensation calibrates four regulatory RNAs to control *Vibrio cholerae* quorum sensing. *EMBO J* **28:** 429–439.

Teng, S.W., Schaffer, J.N., Tu, K.C., Mehta, P., Lu, W., Ong, N.P., *et al*. (2011) Active regulation of receptor ratios controls integration of quorum-sensing signals in *Vibrio harveyi*. *Mol Syst Biol* **7:** 491.

Timmen, M., Bassler, B.L., and Jung, K. (2006) AI-1 influences the kinase activity but not the phosphatase activity of LuxN of *Vibrio harveyi*. *J Biol Chem* **281:** 24398–24404.

Tong, D., Rozas, N.S., Oakley, T.H., Mitchell, J., Colley, N.J., and McFall-Ngai, M.J. (2009) Evidence for light perception in a bioluminescent organ. *Proc Natl Acad Sci USA* **106:** 9836–9841.

Tsou, A.M., Cai, T., Liu, Z., Zhu, J., and Kulkarni, R.V. (2009) Regulatory targets of quorum sensing in *Vibrio cholerae*: evidence for two distinct HapR-binding motifs. *Nucleic Acids Res* **37:** 2747–2756.

Tu, K.C., and Bassler, B.L. (2007) Multiple small RNAs act additively to integrate sensory information and control quorum sensing in *Vibrio harveyi*. *Genes Dev* **21:** 221–233.

Tu, K.C., Long, T., Svenningsen, S.L., Wingreen, N.S., and Bassler, B.L. (2010) Negative feedback loops involving small regulatory RNAs precisely control the *Vibrio harveyi* quorum-sensing response. *Mol Cell* **37:** 567–579.

Urbanowski, M.L., Lostroh, C.P., and Greenberg, E.P. (2004) Reversible acyl-homoserine lactone binding to purified *Vibrio fischeri* LuxR protein. *J Bacteriol* **186:** 631–637.

Visick, K.L., and Ruby, E.G. (2006) *Vibrio fischeri* and its host: it takes two to tango. *Curr Opin Microbiol* **9:** 632–638.

Visick, K.L., Foster, J., Doino, J., McFall-Ngai, M., and Ruby, E.G. (2000) *Vibrio fischeri lux* genes play an important role

in colonization and development of the host light organ. *J Bacteriol* **182:** 4578–4586.

Watson, W.T., Minogue, T.D., Val, D.L., von Bodman, S.B., and Churchill, M.E. (2002) Structural basis and specificity of acyl-homoserine lactone signal production in bacterial quorum sensing. *Mol Cell* **9:** 685–694.

Wier, A.M., Nyholm, S.V., Mandel, M.J., Massengo-Tiasse, R.P., Schaefer, A.L., Koroleva, I., *et al*. (2010) Transcriptional patterns in both host and bacterium underlie a daily rhythm of anatomical and metabolic change in a beneficial symbiosis. *Proc Natl Acad Sci USA* **107:** 2259–2264.

Williams, J.W., Cui, X., Levchenko, A., and Stevens, A.M. (2008) Robust and sensitive control of a quorum-sensing circuit by two interlocked feedback loops. *Mol Syst Biol* **4:** 234.

Wollenberg, M.S., Preheim, S.P., Polz, M.F., and Ruby, E.G. (2011) Polyphyly of non-bioluminescent *Vibrio fischeri* sharing a *lux*-locus deletion. *Environ Microbiol* **14:** 655–668.

Zhang, R.G., Pappas, K.M., Brace, J.L., Miller, P.C., Oulmassov, T., Molyneaux, J.M., *et al*. (2002) Structure of a bacterial quorum-sensing transcription factor complexed with pheromone and DNA. *Nature* **417:** 971–974.

Zhu, J., and Winans, S.C. (2001) The quorum-sensing transcriptional regulator TraR requires its cognate signaling ligand for protein folding, protease resistance, and dimerization. *Proc Natl Acad Sci USA* **98:** 1507–1512.