MicroReview

Shedding light on bioluminescence regulation in Vibrio fischeri

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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–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 (FMNH₂) (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
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. 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.

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.
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 Esal 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 j2 and j3. 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 over-produced 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 descendents 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-oxoctanoyl-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 lux 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-oxoctanoyl-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 mM 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 polyphylly, 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 luxCDABEG 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
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.
Specifically, the luminescence emitted by an parallel LuxS–LuxP/Q system (Lupp and Ruby, 2004). Impact on bioluminescence and host colonization than the attention in 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 Al-2 concentration is low. However, signalling by Al-2 appears to be inconsequential during the initiation of symbiosis by V. fischeri, because a ΔluxS 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 Al-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). Resulting 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 phylogenograms 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, luxO 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, ΔluxO or ΔluxA *V. fischeri* strains (Chun et al., 2008). The presence of the bacterial symbionts (wild type, ΔluxO or ΔluxA) 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 ΔluxO or ΔluxA 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 respond 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 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.

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* (Engberbrecht 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 *luxG* 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...
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.

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References


