

# Biochemistry and Genetics of Bacterial Bioluminescence

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**Abstract** Bacterial light production involves enzymes—luciferase, fatty acid reductase, and flavin reductase—and substrates—reduced flavin mononucleotide and long-chain fatty aldehyde—that are specific to bioluminescence in bacteria. The bacterial genes coding for these enzymes, *luxA* and *luxB* for the subunits of luciferase; *luxC*, *luxD*, and *luxE* for the components of the fatty acid reductase; and *luxG* for flavin reductase, are found as an operon in light-emitting bacteria, with the gene order, *luxCDABEG*. Over 30 species of marine and terrestrial bacteria, which cluster phylogenetically in *Aliivibrio*, *Photobacterium*, and *Vibrio* (*Vibrionaceae*), *Shewanella* (*Shewanellaceae*), and *Photorhabdus* (*Enterobacteriaceae*), carry *lux* operon genes. The luminescence operons of some of these bacteria also contain genes involved in the synthesis of riboflavin, *ribEBHA*, and in some species, regulatory genes *luxI* and *luxR* are associated with the *lux* operon. In well-studied cases, *lux* genes are coordinately expressed in a population density-responsive, self-inducing manner called quorum sensing. The evolutionary origins and physiological function of bioluminescence in bacteria are not well understood but are thought to relate to utilization of oxygen as a substrate in the luminescence reaction.

**Keywords** Bioluminescence • Bacterial luciferase • *Aliivibrio* • *Photobacterium* • *Vibrio* • *lux* genes

## Abbreviations

acyl-HSL	Acyl-homoserine lactone
FMNH <sub>2</sub>	Reduced flavin mononucleotide
Kb	Kilobases (thousand nucleotides)
kD	Kilodaltons

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Mb	Megabases (million nucleotides)
RCHO	Long-chain fatty aldehyde
RCOOH	Long-chain fatty acid

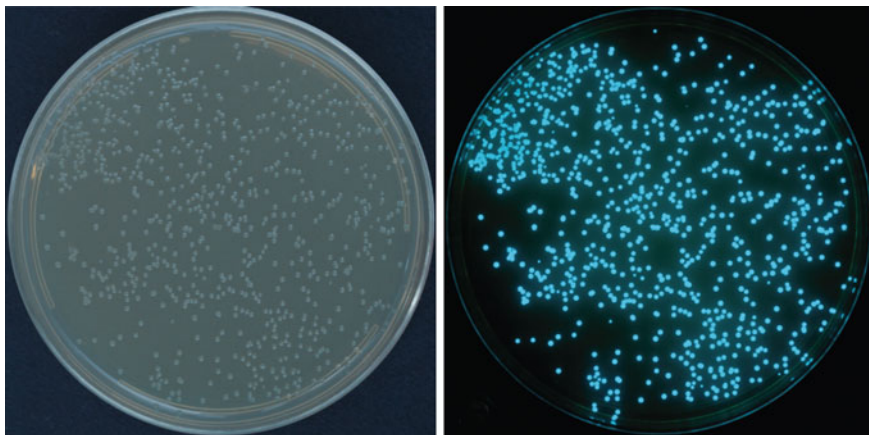
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## 1 Introduction

Light production by bacteria is one of several evolutionarily distinct kinds of bioluminescence, other kinds of which are found in various terrestrial and marine eukaryotic organisms [76, 199]. Bacterial bioluminescence has been known since 1875, when Pflüger [152] correlated the presence of bacteria in the surface slime of a fish with luminescence [74, 159]; earlier observations of luminescence, during the 1700 and 1800s, from various sources were likely due similarly to the presence of luminous bacteria, such as saprophytes or parasites [72, 73]. The oxygen-dependence of bacterial luminescence, first revealed by Boyle [24], who showed that light produced by decaying fish required air, suggests light production in bacteria arose evolutionarily after oxygen levels began to rise through the activities of early cyanobacteria, oxygenic phototrophs, approximately 2.4 billion years ago.

All luminous bacteria, as far as known to date, utilize the same enzymatic reaction for light production, based on bacterial luciferase. Phylogenetic analysis suggests that the genes coding for the bacterial luminescence enzymes arose once evolutionarily. Currently, luminous bacteria are grouped primarily in one *Gammaproteobacteria* family, *Vibrionaceae*. Some luminous members of closely related families exist, however, having apparently acquired the genes for luminescence by horizontal gene transfer. Historical perspectives on the first isolations and analyses and early taxonomy of luminous bacteria link the study of these bacteria to the origins of general microbiology [44, 57, 73, 159]. This chapter



**Fig. 1** Bacterial luminescence. Colonies of *P. mandapamensis* from the light organ of the cardinalfish *Siphamia tubifer* (Perciformes: Apogonidae) are shown growing on a nutrient seawater agar plate. The plate was photographed in room light (*left*) and (the same plate) in the dark by the light produced by the bacteria (*right*). From Dunlap et al. [46]

outlines the current systematics of luminous bacteria, provides an overview of the biochemistry and genetics of bacterial luminescence, and concludes with a discussion of the evolutionary origin and function of bacterial luminescence.

## 2 Species of Luminous Bacteria

Thirty or more species of bacteria have strains that make light visible to the eye (Fig. 1) or that at least carry genes for luminescence, the *lux* genes (Table 1). These bacteria, which are all Gram-negative, group phylogenetically as members of three families of *Gammaproteobacteria*: *Vibrionaceae* (*Aliivibrio*, *Photobacterium*, *Vibrio*), *Enterobacteriaceae* (*Photorhabdus*), and *Shewanellaceae* (*Shewanella*). It should be noted, however, that most *Vibrionaceae* species are not luminous and apparently lack *lux* genes and that only the few listed species in *Enterobacteriaceae* and *Shewanellaceae* are known to be luminous. Also, many of the species listed have nonluminous strains that may or may not lack *lux* genes [4, 19, 38, 60, 204]. The low number of luminous species, relative to the many described species in *Vibrionaceae*, is consistent with loss of the *lux* genes over evolutionary time from the ancestors of many lineages within the family. Furthermore, nonluminous variants apparently can arise readily through the loss of one of more core genes of the *lux* operon, *luxCDABEG* (e.g., [204]). It should be noted here also that several species previously classified variously as members of *Photobacterium* or *Vibrio* (i.e., *fischeri*, *salmonicida*, *logei*, and *wodanis*) have been reclassified as members of

**Table 1** Species and ecological sources of luminous bacteria

Species	Sources	Selected references
<b>Marine</b>		
<i>Aliivibrio</i>		
<i>fischeri</i>	Coastal seawater, light organs of squid and fish	[22, 59, 157, 161, 163, 164, 188]
<i>logei</i>	Coastal seawater, sediment	[9, 12, 188]
<i>salmonicida</i>	Tissue lesions of Atlantic salmon	[91, 139, 188]
<i>sifiae</i>	Coastal seawater	[9, 209]
“ <i>thorii</i> ”	Light organs of squid	[9, 54]
<i>wodanis</i>	Coastal seawater, diseased farmed salmon, light organs of squid	[9, 115, 188]
<i>Photobacterium</i>		
<i>aquimaris</i>	Coastal seawater	[208]
<i>damselae</i>	Coastal seawater	[173, 189]
<i>kishitanii</i>	Light organs and skin of fish	[6, 7]
<i>leiognathi</i>	Coastal seawater, light organs of fish	[23, 45, 65, 158]
<i>mandapamensis</i>	Coastal seawater, light organs of fish	[85, 93, 157, 191]
<i>phosphoreum</i>	Coastal and pelagic seawater	[6, 7, 25, 202]
<i>Candidatus Photodesmus katoptron</i>	Light organs of <i>Anomalops katoptron</i>	[80, 86–88, 203]
<i>blepharon palpebratus</i>	Light organs of <i>Photobelpharon palpebratus</i>	[80, 87, 88, 203]
<i>Shewanella</i>		
<i>hanedai</i>	Seawater and sediment	[92]
<i>woodyi</i>	Seawater and squid ink	[117]
<i>Vibrio</i>		
<i>azureus</i>	Coastal seawater	[207]
<i>campbellii</i>	Coastal seawater	[107, 175]
<i>chagasii</i>	Coastal seawater, surfaces and intestines of marine animals	[182, 189]
<i>harveyi</i>	Coastal seawater, sediment	[69, 142, 157, 163, 206]
<i>jascicida</i>	Coastal seawater	[57, 193]; H. Urbanczyk (pers. comm.)
<i>mediterranea</i> <sup>a</sup>	Coastal seawater	[153]
<i>orientalis</i>	Seawater, surface of shrimp	[205]
<i>owensii</i>	Coastal seawater	[193]; H. Urbanczyk (pers. comm.)
<i>sagamiensis</i>	Coastal seawater	[210]
<i>splendidus</i>	Coastal seawater	[20, 138]
<i>vulnificus</i>	Coastal seawater, oysters	[147, 189]
<b>Brackish/Estuarine</b>		
<i>Vibrio</i>		
<i>cholerae</i>	Estuaries, bays, coastal seawater	[93, 148, 154, 215]
<b>Terrestrial</b>		

(continued)

**Table 1** (continued)

Species	Sources	Selected references
<i>Photorhabdus</i>		
<i>asymbiotica</i>	Human skin lesions	[53, 58, 99, 149, 200]
<i>luminescens</i>	Insect larvae infected with heterorhabditid nematodes	[21, 36, 58, 181]
<i>temperata</i>	Insect larvae infected with heterorhabditid nematodes	[58, 181]

<sup>a</sup> Ability of this species to luminescence has not been confirmed; the single strain reported as luminous [153] might not be available

*Aliivibrio*, a change that resolves long-standing confusion on the evolutionary relationships of these bacteria with members of *Vibrio* and *Photobacterium* [9, 188].

The majority of the luminous bacteria, members of *Aliivibrio*, *Photobacterium*, *Vibrio*, and *Shewanella*, are found in the marine environment. Luminous strains of *Vibrio cholerae* can be isolated from brackish environments and freshwater as well as from coastal seawater. Depending on the species, these bacteria occur free in seawater and in sediments or more commonly are associated with surfaces and gut tracts of marine animals as saprophytes and commensal symbionts (e.g., [160, 190]). They also occur as parasites of marine animals and as highly specific mutualistic bioluminescent light organ symbionts of many marine fish and squid. In contrast, *Photorhabdus* species occur in terrestrial environments and as symbionts of terrestrial heterorhabditid nematodes [195]. Several of the species listed in Table 1 were described recently, and others have been recognized only recently as luminous or as carrying *lux* genes. This progress suggests that future studies, especially those employing whole genome sequence analysis (e.g., [193]), will identify many more species and strains of bacteria that are luminous or at least carry *lux* genes.

Most luminous bacteria are culturable on laboratory media. Some, however, have not yet been brought into culture despite many attempts [81]. These not-yet cultured luminous bacteria are symbiotic in the subocular light organs of flashlight fish (Anomalopidae) and the escal light organs of members of many families of deep-sea anglerfish (order Lophiiformes). The inability to culture these bacteria suggests they are obligately dependent on their host fish for nutrients necessary for reproduction or conditions supporting their survival [81]. Early phylogenetic work placed these bacteria in *Vibrionaceae* and as a species distinct from the luminous bacteria known at the time [80, 81, 83, 84]. Recent analyses of the bacteria from light organs of two species of anomalopid fish, using multilocus phylogenetic analysis and whole genome sequence analysis, have identified the bacteria as members of a new *Vibrionaceae* genus, *Candidatus Photodesmus*, with two new species, *Candidatus Photodesmus katoptron* and *Candidatus Photodesmus blepharon*, from *Anomalops katoptron* and *Photoblepharon palpebratus*, respectively. The genomes of *Ca. Photodesmus katoptron* and *Ca. Photodesmus blepharon*, approximately 1.0 and

1.1 Mb, respectively, are massively reduced compared to the genomes of other members of *Vibrionaceae*, which typically are 4.5–6.0 Mb, with many metabolic and regulatory genes lost. Genome reduction and gene loss apparently account for the inability of these bacteria to grow in laboratory culture [86–88]. For the deep-sea anglerfish, no additional information is available at this time on the species-level phylogenetic placement of their symbiotic luminous bacteria.

Despite much recent progress in clarifying the taxonomy of luminous bacteria (Table 1), the ecological incidence and species-level diversity of luminous bacteria remain incompletely defined. One problem is that the luminescence phenotype can be lost; strains luminous on primary isolation often become dim or dark in laboratory culture [3, 134, 171]. Another is that some species that grow well in laboratory culture at room temperature, i.e., *Aliivibrio logei* and *Shewanella hanedai*, typically produce light visible to the eye only when grown at cooler temperatures. In some cases, i.e., luminous bacteria infecting crustaceans; [67] and strains of *Aliivibrio fischeri* symbiotic with the Hawaiian sepiolid squid, *Euprymna scolopes* [22], the bacteria produce a high level of light in their natural habitat but produce little or no light when grown in laboratory culture. A further complication is that strains of some bacteria, such *V. cholerae*, are known to carry *lux* genes but apparently do not express them (e.g., [93, 148, 154, 215]). In addition, bacteria identified as related to *Vibrio harveyi* and *Vibrio cincinnatiensis* carry the *lux* genes but have been found to have *lux* gene mutations that result in a dark phenotype [143]. Although the phylogenetically scattered incidence of bacteria with *lux* genes in *Vibrionaceae* presumably relates to different ecologies of the different species, it is not obvious how having and expressing *lux* genes contributes to the lifestyle of most luminous bacteria; there are no obvious ecological differences between luminous and nonluminous species except in the case of those species that are bioluminescent symbionts of bacterially luminous fish and squid. On-going and future studies that examine the incidence, *lux* gene content, and phylogenetic placement of luminous bacteria will undoubtedly expand understanding of the species diversity and ecology of bacteria able to produce light.

### 3 Biochemistry of Bacterial Luminescence

Light emission in bacteria is catalyzed by a uniquely bacterial kind of luciferase, a heterodimeric protein of approximately 80 kD, composed of  $\alpha$  (40 kDa) and  $\beta$  (37 kDa) subunits, with homology to long-chain alkane monooxygenases [76, 104]. The enzyme mediates the oxidation of reduced flavin mononucleotide (FMNH<sub>2</sub>) and a long-chain aliphatic (fatty) aldehyde (RCHO) by O<sub>2</sub> to produce blue-green light according to the following reaction.

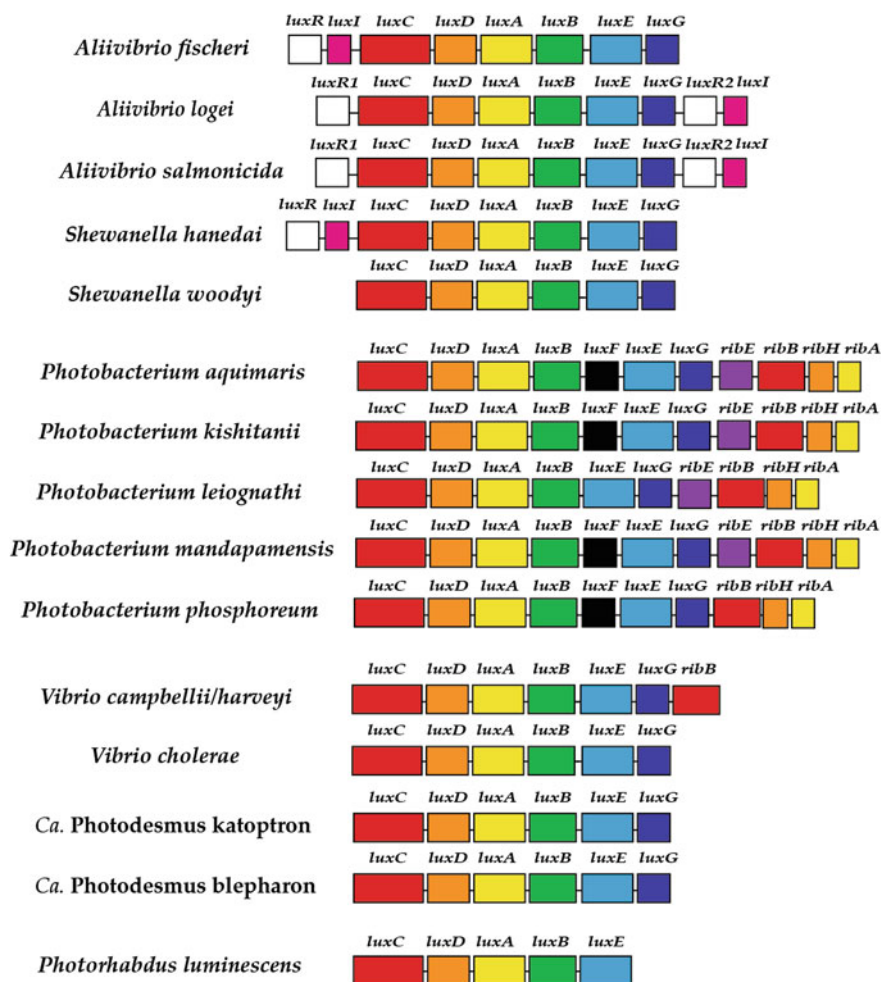


Along with bacterial luciferase, the substrates, FMNH<sub>2</sub> and long-chain fatty aldehyde, are specific to the bacterial luminescence reaction; bioluminescent eukaryotes employ different chemistries and luciferases that are not homologous at the protein or gene sequence levels to bacterial luciferase [76]. In the luminescence reaction, binding of FMNH<sub>2</sub> by the enzyme is followed by interaction with O<sub>2</sub> to form a flavin-4a-hydroperoxide. Association of this complex with aldehyde forms a highly stable intermediate, the slow decay of which results in oxidation of the FMNH<sub>2</sub> and aldehyde substrates and the emission of light [76, 79]. Quantum yield for the reaction has been estimated at 0.1–0.2 photons. The reaction is highly specific for FMNH<sub>2</sub>, and the aldehyde substrate *in vivo* is likely to be tetradecanal. FMNH<sub>2</sub> is provided by the activity of an NADH:FMN oxidoreductase (flavin reductase), which taps reductant from NADH generated in cellular metabolism, for example, glycolysis and the citric acid cycle. Transfer of reductant from FMNH<sub>2</sub> to luciferase occurs by free diffusion. Synthesis of the long-chain aldehyde is catalyzed by a fatty-acid reductase complex composed of three polypeptides, an NADPH-dependent acyl protein reductase (r, 54 kDa), an acyl transferase (t, 33 kDa), and an ATP-dependent synthetase (s, 42 kDa). The complex has a stoichiometry of r<sub>4</sub>s<sub>4</sub>t<sub>2–4</sub>, and its activity is essential for the production of light in the absence of exogenously added aldehyde [79, 121, 183, 201]. Luciferases from different species of luminous bacteria exhibit substantial amino acid residue and nucleotide sequence identity [45, 122], consistent with a common evolutionary origin of luminescence in bacteria.

## 4 Bacterial *lux* Genes

The *lux* operon, *luxCDABEG*, contains the genes necessary for light production in bacteria (Fig. 2). The *luxA* and *luxB* genes code for the  $\alpha$  and  $\beta$  subunits of bacterial luciferase, respectively, *luxC*, *luxD*, and *luxE* genes, respectively, code for the r, s, and t polypeptides of the fatty-acid reductase complex that synthesizes and recycles aldehyde substrate for luciferase, and *luxG*, codes for flavin reductase [112, 122, 141, 178, 183]. The absence of *luxG* from the *lux* operons of *Photobacterium luminescens* and newly characterized species of *Ca. Photodesmus* (Fig. 2) apparently is compensated for by the activity of a flavin reductase activity coded for by an *Escherichia coli* *fre*-like gene. Homologues of the *fre*-like gene are found in various luminous bacteria [212–214].

An additional gene, *luxF*, which codes for a nonfluorescent flavoprotein, is present in the *lux* operons of *Photobacterium*, between *luxB* and *luxE* (Fig. 2). The LuxF protein might function in the luminescence system by scavenging an inhibitory side product of the luciferase reaction [130], but it is not necessary for light production, even in those *Photobacterium* species that normally carry this gene [93]. The *luxF* gene apparently has been secondarily lost in *Photobacterium leiognathi* [5].



**Fig. 2** Bacterial luminescence (*lux*) genes. Shown are the gene content and gene order of *lux* operons for those bacteria for which complete *lux* operon sequence data are available. Contiguous genes of the *lux* operons are aligned to highlight commonalities and differences. Four distinct types of *lux* operons are evident based on commonalities of gene content, organization, and sequence similarity: (1) *Aliivibrio/Shewanella* type, with *luxI/luxR* regulatory genes; (2) *Photobacterium* type, with *ribEBHA* genes forming a *lux-rib* operon; (3) *Vibrio/Candidatus Photodesmus* type, without linked regulatory genes; and (4) *Photorhabdus* type, composed of just five core *lux* genes, *luxCDABE*. Additional species of luminous bacteria are listed in Table 1. See text for details and for information on accessory genes

In *Photobacterium*, genes involved in the synthesis of riboflavin, *ribEBHA*, are part of the *lux* operon, forming a *lux-rib* operon, *luxCDABFEG-ribEBHA* (Fig. 2; [8, 100, 101, 113, 176]). The absence of a transcriptional stop or other regulatory site between the *lux* and *rib* genes indicates that these genes are coordinately

expressed from a single promoter upstream of *luxC*. Strains of *P. phosphoreum* lack *ribE*, which presumably was lost in the divergence from an ancestral *Photobacterium* that gave rise to this species. The presence of genes for synthesis of riboflavin as part of the *lux* operon might enhance light production by ensuring coordinate synthesis of luciferase and substrates for the enzyme. The *lux* operon of *V. campbellii* (previously classified as *V. harveyi*; [107]) contains *ribB*, coding for 3,4-dihydroxy-2-butanone 4-phosphate synthase, a key enzyme in riboflavin synthesis (referred to originally as *luxH*; [179]). In *A. fischeri*, although *ribB* is not part of the *lux* operon, its expression nonetheless is under the same regulatory control as the *lux* genes [26].

The isolation of a luminous strain of *V. campbellii* was first reported several years ago [175]. More recently, certain strains identified as *V. harveyi*, for example, BAA-1116 (a.k.a. BB120, which has been used extensively in studies of quorum sensing, e.g., [15]), were identified by whole genome sequence analysis as members of *V. campbellii* [107]. Careful phylogenetic analysis of strains currently referred to as *V. harveyi* is therefore needed to confirm their identity and the *lux* operon gene organization of this species.

In *Photobacterium*, many strains of *P. leiognathi* carry two intact and apparently functional *lux-rib* operons in their genomes [8]. The two operons, *lux-rib*<sub>1</sub> and *lux-rib*<sub>2</sub>, are distinct in sequence and genomic location. Phylogenetic analysis indicates that *lux-rib*<sub>1</sub> and *lux-rib*<sub>2</sub> are more closely related to each other than either is to the *lux* and *rib* genes of other bacterial species [8]. These findings indicate that *lux-rib*<sub>2</sub> did not arise by a gene duplication event, and they exclude interspecies horizontal transfer as the origin of *lux-rib*<sub>2</sub> in *P. leiognathi*; instead, the second operon apparently was acquired by horizontal gene transfer from a lineage of *P. leiognathi* that either has gone extinct or has not yet been sampled. The *P. leiognathi lux-rib*<sub>2</sub> operon has also been found in two strains of *P. mandapamensis*, which also carry a normal *P. mandapamensis lux-rib* operon, and in a strain of *P. damsela*, a species not previously known to be luminous [189]. In *Photobacterium aquimaris* [208], the *lux* operon of one of two luminous strains apparently has been replaced by horizontal acquisition of the *lux* operon of *Photobacterium mandapamensis* [192].

In addition to the presence of the *ribEBHA* genes as part of the *lux* operon of *Photobacterium*, other genus-specific differences are evident in the genes upstream of and flanking the *lux* operons of luminous bacteria (Fig. 2). In *P. mandapamensis*, the *lux-rib* operon is preceded by *lumQ* and *lumP*, which form the lumazine operon. The function of *lumQ* is not yet known, although it is thought to code for a DNA-binding protein [109]. LumP, a 21-kDa fluorescent accessory protein referred to as lumazine protein, functions to enhance the intensity of light emission and to shift the emission wavelength of luciferase from blue-green (495 nm) to blue (475–486 nm) [102, 145, 146, 151]. A *lumP* gene is present just upstream of *luxC* in *Photobacterium phosphoreum* and *Photobacterium aquimaris*, but *lumQ* is absent [192]. The LumP protein, which has been isolated from *P. phosphoreum* and from a strain of *P. mandapamensis* (previously classified as a strain of *P. leiognathi*) and also purified from *P. kishitanii*, contains a noncovalently bound

fluorophore, 6,7-dimethyl-8-ribityllumazine, the immediate biosynthetic precursor of riboflavin [145, 165, 172]. In *P. leiognathi* *lumP* is not found, although approximately 200 nucleotides of the *P. leiognathi luxC-lumQ* intergenic region can be aligned to the *P. mandapamensis lumP* gene [8]. The activity of the LumP protein apparently accounts for the blue-shifted luminescence of *P. mandapamensis* compared to *P. leiognathi*, one of the diagnostic traits distinguishing these two species [5, 102, 145, 146, 151, 93]. The genes flanking the *P. leiognathi* and *P. mandapamensis lux-rib* operons are homologous to a single contiguous region in nonluminous *P. angustum* [8, 108–111, 113].

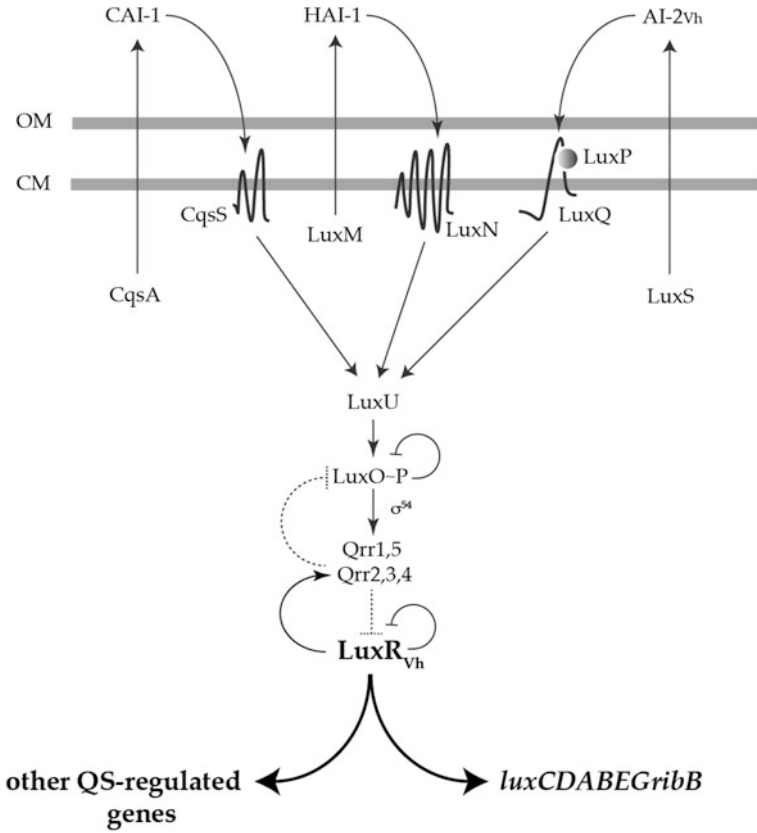
In the examined *Aliivibrio* species, regulatory genes, *luxI* and *luxR*, which control transcription of the *lux* operon (described below), are upstream of or flank the *luxCDABEG* genes (Fig. 2). The *luxI* gene codes for an acyl-homoserine lactone (acyl-HSL) synthase [166], and *luxR* codes for a receptor protein that interacts with acyl-HSL to activate transcription of the *lux* operon [52]. In *Aliivibrio fischeri*, *luxI* is the first gene of the *lux* operon, and *luxR*, upstream of *luxI*, is divergently transcribed (Fig. 2). The *lux* operon of *Shewanella hanedai* has the same gene arrangement. This similarity, together with a high degree of *lux* gene sequence identity suggests that *S. hanedai* acquired its *lux* operon by horizontal transfer from *A. fischeri* or the ancestor of *A. fischeri* [189]. In *Aliivibrio salmonicida*, a bacterium that requires exogenous addition of aldehyde to produce a high level of light [55], two *luxR* genes, homologous to *A. fischeri luxR*, flank the *lux* operon; a *luxI* gene also is present, divergently transcribed from the downstream *luxR* (Fig. 2; [91, 139]). The same arrangement of *luxI* and *luxR* genes as in *A. salmonicida* is present in *Aliivibrio logei* [119]. In contrast to *A. salmonicida*, however, exogenous addition of aldehyde is not required for a high level of light production in *A. logei* [119]; mutations in *luxD* account for the exogenous aldehyde requirement of *A. salmonicida*. Genes flanking the *lux* operons of other luminous *Aliivibrio* species (Table 1) apparently have not yet been characterized. An accessory protein, yellow fluorescent protein (YFP), is present in *Aliivibrio sifiae* (previously referred to as *A. fischeri*; [9, 209] and shifts the emission wavelength of luminescence toward yellow [11, 33, 150, 162]; the YFP gene apparently is not linked to the *lux* operon.

With respect to *S. hanedai* and *S. woodyi*, comparison of genes flanking the *lux* operons suggested that these species had acquired *lux* genes from a member of *Aliivibrio* [96], a possibility confirmed through phylogenetic analysis [189]. In *Photorhabdus* species, the *luxCDABE* genes also might have been acquired by horizontal gene transfer, possibly from an ancestor of *V. harveyi* [61, 121, 123]. Phylogenetic analysis of the *Photorhabdus lux* genes, however, neither supports nor contradicts horizontal acquisition of the *lux* genes by *Ph. luminescens* [189]. Certain instances of horizontal acquisition of *lux* genes have also been found for *Vibrio* [189]. The one known luminous strain of *Vibrio vulnificus*, the human pathogen [147], apparently acquired its *lux* genes from *V. harveyi*, and in *V. chagasii*, a species not described as luminous [182], two luminous strains were identified and apparently had acquired their *lux* genes from *V. harveyi* and *V. splendidus*, respectively [189].

The arrangement of genes associated with *luxCDABEG* of the examined *Vibrio* species differs substantially from that in *Photobacterium* and in *Aliivibrio* (Fig. 2). Regulatory genes controlling transcription of the *lux* operon are not part of and are not adjacent to the *lux* operons of those *Vibrio* species examined; specifically, a *luxR* gene, referred to here as *luxR<sub>vh</sub>* which is not homologous to *A. fischeri luxR*, is not physically associated with the *lux* operon in *V. campbellii* (*V. harveyi*). Also, with the exception of *ribB* in *V. campbellii*, genes involved in riboflavin synthesis in *Photobacterium*, *ribE*BHA, are not part of the *lux* operon in *Vibrio*. Conservation of *luxCDABEG* as a unit might reflect a need for close interaction of luciferase with the enzymes, fatty acid reductase and flavin reductase, producing substrates for the reaction, for efficient light production. However, it is not obvious what led to the genus-specific *lux* operon gene content and organization in *Aliivibrio*, *Photobacterium*, and *Vibrio* (Fig. 2), three closely related genera of *Vibrionaceae*.

## 5 Regulation of *lux* Operon Expression

The production of light consumes a substantial amount of energy, through the synthesis and activity of the Lux proteins [41]. The retention and expression of *lux* genes in many bacteria, despite this energetic cost, therefore indicates that the activity of the luminescence system must benefit these bacteria, physiologically or ecologically, as discussed below. Furthermore, this energetic cost presumably accounts for the population density-responsive regulation of *lux* operon expression characteristic of many luminous bacteria. Originally called autoinduction and discovered through study of the pattern of luminescence and luciferase synthesis of *V. harveyi* in batch culture [132, 136], this gene regulatory mechanism is now referred to as quorum sensing to reflect its relationship with population density [66, 70, 77, 124]. At low population density, very little luciferase is synthesized, and consequently little light is produced, whereas at high population density, luciferase levels are induced 100–1,000-fold and light levels increase by  $10^3$ – $10^6$ -fold. This population density-responsive induction of luciferase synthesis and luminescence is controlled in part by the production and accumulation in the cell's local environment of small secondary metabolite signal molecules, called autoinducers, which function via regulatory proteins to activate or derepress transcription of the *lux* operon. Quorum sensing has been studied intensively in two luminous bacteria, *V. harveyi* and *A. fischeri*. With respect to *V. harveyi*, a strain used extensively in quorum-sensing research, BAA-1116, also known as BB120 (e.g., [15]), recently was recognized through whole genome sequence analysis to be a member of *V. campbellii* [107]. The information related below is therefore provisionally ascribed to *V. campbellii*, pending resolution of the taxonomic status of strains called *V. harveyi* that have been used in the various studies of quorum sensing. The quorum-sensing systems of these two bacteria, *V. campbellii* and *A. fischeri*, briefly outlined below, differ substantially at genetic and chemical levels. Despite the many



**Fig. 3** Quorum-sensing regulatory circuitry in *Vibrio campbellii* (*Vibrio harveyi*). The expression of *lux* operon, and of other quorum-sensing-regulated genes, in *V. campbellii* (previously classified as *V. harveyi*; [107]) is coordinated by three chemically distinct autoinducers, HAI-1, AI-2<sub>vh</sub>, and CAI-1, that modulate the phosphorylation state of *luxU*. The synthesis of each autoinducer is catalyzed by a different protein, LuxM, LuxS, and CqsA, and each is recognized by a different cytoplasmic membrane-associated two-component histidine-kinase receptor, LuxN, LuxPQ, and CqsS, respectively. Low concentrations of the autoinducers lead to phosphorylation of LuxO and via quorum-regulatory RNAs to the destabilization of the *luxR<sub>vh</sub>* transcript, thereby blocking *lux* operon transcriptional activation by LuxR<sub>vh</sub>. High concentrations of the autoinducers reverse the phosphorylation cascade, allowing formation of LuxR<sub>vh</sub> and activation of *lux* operon transcription. Arrows indicate positive interactions or transcriptional activation, whereas bars indicate negative interactions or blocking of transcription. See the text for details and references. Redrawn from [185]

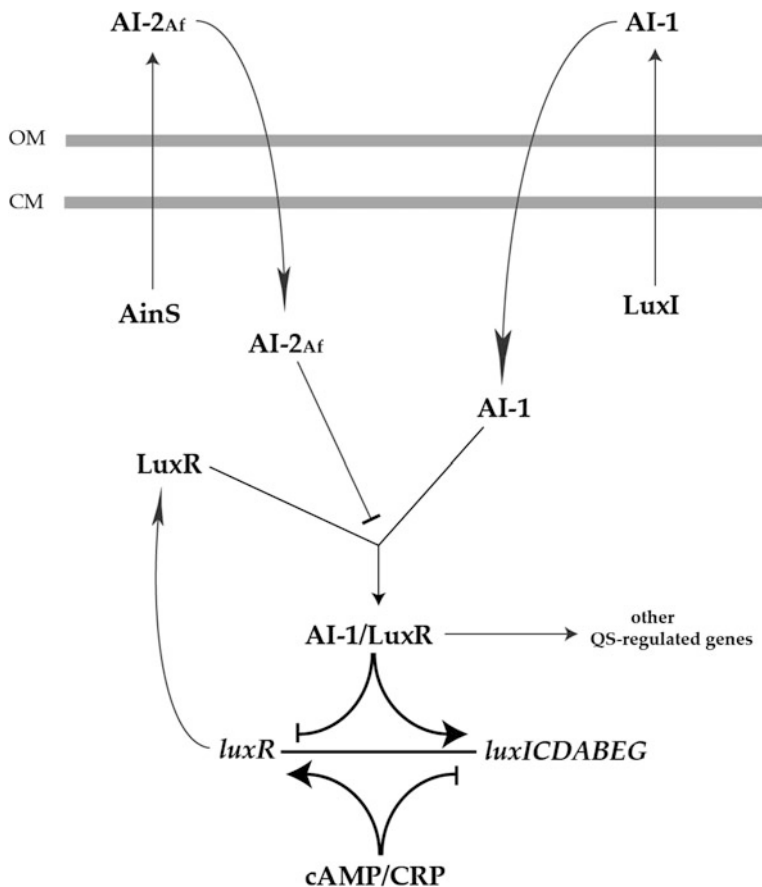
differences, however, several commonalities to the two systems have been identified [44]. Recent publications provide additional details on quorum sensing in these bacteria as well as in nonluminescent bacteria [14, 44, 155].

In *V. campbellii*, *lux* operon expression is controlled by a multicomponent phosphorylation/dephosphorylation cascade (Fig. 3). Three chemically distinct autoinducers are involved: 3-hydroxybutanoyl-HSL (*harveyi* autoinducer-1,

HAI-1), (2S,4S)-2-methyl-2,3,3,4-tetrahydroxytetrahydrofuran borate (*V. harveyi* autoinducer-2, AI-2<sub>Vh</sub>), and (S)-3-hydroxytridecan-4-one (cholerae autoinducer, CAI-1) [27, 31, 90]. Synthesis of HAI-1 is dependent on LuxM [16], synthesis of AI-2<sub>Vh</sub> is catalyzed by LuxS [167], and synthesis of CAI-1 is catalyzed by CqsA [97, 198]. Each of these molecules is recognized by a different cytoplasmic membrane-associated two-component histidine-kinase receptor, LuxN [16, 64], LuxPQ [18], and CqsS [89], respectively. When concentrations of the autoinducers are low, such as at low population density or in habitats in which the autoinducers diffuse away rapidly from cells (i.e., in seawater), the receptor proteins function as kinases, transferring phosphate to LuxU, a histidine-phosphotransfer protein. LuxU then transfers the phosphate to LuxO, a DNA-binding response regulator, the expression of which is subject to repression by LuxT [13, 17, 27, 62, 63, 106, 114, 126, 177, 197]. LuxO ~ P, together with sigma factor  $\sigma^{54}$ , then activates the expression of genes coding for five small quorum-regulatory RNAs (Qrr), Qrr1 through Qrr5 [103, 184]. The Qrr RNAs bind and destabilize the *luxR<sub>Vh</sub>* transcript, blocking production of LuxR<sub>Vh</sub> protein, the transcriptional activator of the *lux* operon [170, 180], and thereby preventing activation of *lux* operon transcription. Conversely, once autoinducer concentrations have attained high levels in the cell's local environment, they bind to their receptors, causing the receptors to switch from kinases to phosphatases, leading to the dephosphorylation of LuxO. With the resulting cessation of *qrr* gene transcription, a *luxR<sub>Vh</sub>* message is produced and translated, and LuxR<sub>Vh</sub> activates *lux* operon transcription. Negative autoregulation of LuxR<sub>Vh</sub> adds additional complexity to this system [28, 125], as does the negative autoregulation of LuxO, posttranscriptional control of LuxO by Qrr sRNAs [185], and involvement of 3':5'-cyclic AMP (cAMP) and camp receptor protein (CRP) [29, 30, 34, 127, 137, 187]. The complexity of this regulatory system apparently benefits *V. campbellii* by allowing a fine-tuning of its quorum-sensing response to differences in the various habitats the bacterium colonizes [140, 185, 196].

In *A. fischeri*, quorum-sensing control of luminescence in *A. fischeri* involves a population-density-dependent accumulation of the autoinducer 3-oxo-hexanoyl-homoserine lactone (AI-1), a membrane-permeant molecule that triggers induction of *lux* operon transcription when AI-1 reaches a critical concentration (Fig. 4). Synthesis of AI-1 is catalyzed by LuxI, an acyl-homoserine lactone synthase, from S-adenosyl methionine and acyl-HSL. The regulatory genes, *luxR<sub>Af</sub>* and *luxI*, are directly linked to the *lux* operon. The *luxR<sub>Af</sub>* gene, which is upstream of the *lux* operon and divergently transcribed from it, encodes a transcriptional activator protein, LuxR<sub>Af</sub>, which associates with AI-1, forms a complex that binds at a site in the *lux* operon promoter and facilitates the binding of RNA polymerase, thereby activating transcription of the genes for light production, *luxICDABEG*. Because *luxI* is a gene of the *lux* operon, increased transcription leads to increased production of LuxI protein and increased synthesis of AI-1, in an autocatalytic, positive feedback manner. The result is a rapid and strong induction of luciferase synthesis and luminescence [48, 50–52, 94, 166, 174].

Several other regulatory factors modulate quorum-sensing in *A. fischeri*. These factors include: cAMP and CRP, which activate transcription of *luxR<sub>Af</sub>* and



**Fig. 4** Quorum-sensing control of luminescence in *A. fischeri*. The expression of the *lux* operon, and of other quorum-sensing-regulated genes, in *A. fischeri* is mediated primarily by the concentration of AI-1, which forms a complex with LuxR<sub>Af</sub>. Synthesis of AI-1 is dependent on LuxI, and the AI-1/LuxR<sub>Af</sub> complex activates *luxICDABEG* transcription. Together with cAMP, the CRP protein activates expression from the *luxR*<sub>Af</sub> promoter, increasing synthesis of LuxR<sub>Af</sub> and potentiating the system to be induced once sufficient AI-1 has accumulated. The presence of *luxI*, coding for AI-1 synthase, as part of the *lux* operon, leads to increased expression from the *lux* operon promoter, stimulating AI-1 synthesis in an autocatalytic, positive feedback manner; the result is a rapid and strong induction of luciferase synthesis once a threshold concentration of AI-1 is attained. A second autoinducer, AI-2<sub>Af</sub> interacts with LuxR<sub>Af</sub>, interfering with the interaction between AI-1 and LuxR<sub>Af</sub>. The hypothesized AI-2<sub>Af</sub>/LuxR<sub>Af</sub> complex is thought to be transcriptionally less effective and therefore to function to delay the onset of AI-1/LuxR<sub>Af</sub> activation of *luxICDABEG* transcription. See the text for details and references

thereby potentiate the cell's response to AI-1 while repressing transcription of *luxICDABEG*; negative autoregulation of *luxR*<sub>Af</sub> expression by LuxR<sub>Af</sub>/AI-1; a second autoinducer, octanoyl-HSL (AI-2<sub>Af</sub>), which is synthesized by AinS and that interacts with LuxR<sub>Af</sub> apparently interfering with AI-1 binding and thereby

delaying *lux* operon induction; involvement of GroEL in production of active LuxR<sub>AF</sub>; a homologue of the *V. harveyi* LuxO, which functions in *A. fischeri* as a repressor of luminescence apparently in a *qrr*-dependent manner; LitR, a protein with substantial sequence similarity to LuxR<sub>AF</sub> and which positively regulates *lux* operon expression; and an involvement of Fnr and LexA in *lux* operon expression [1, 2, 35, 37, 39, 40, 42, 43, 49, 56, 68, 71, 98, 126, 128, 129, 131, 169, 186]. As in *V. campbellii*, the complexity of inputs into the quorum-sensing regulatory circuitry in *A. fischeri* indicates both a tight integration of luminescence into the lifestyle of the bacterium and the ability to modulate *lux* operon expression in response to a variety of conditions.

## 6 Origin and Function of Luminescence in Bacteria

The conserved gene content and gene order of the *lux* operon in bacteria, *lux-CDABEG* (Fig. 2), and the high levels of *lux* gene and Lux protein amino acid sequence identities among luminous bacteria (e.g., [122]) indicate that all known bacterial *lux* operons derive from a single common ancestor. The congruence of phylogenies based on *lux* genes and other protein coding genes (and the 16S rRNA gene) [189] indicates that bacterial luminescence arose within the *Vibrionaceae* lineage and mostly likely in the ancestor that gave rise to *Aliivibrio*, *Photobacterium*, and *Vibrio*.

The homology of bacterial luciferase to long-chain alkane monooxygenases [104] suggests that the light-emitting enzyme arose from this family of proteins, even though none of the other enzymes in the family emit light [76]. With some light emission, bacteria luciferase might have evolved under ecological selection, according to the following scenario [168]. A flavoprotein catalyzing fatty acid  $\alpha$ -oxidation reactions with low chemiluminescent quantum yields is postulated to have mutated under hypoxic conditions to accept FMNH<sub>2</sub> as the flavin cofactor, generating a fortuitously high fluorescence yield, termed “protobioluminescence,” via the 4a-hydroxy-FMNH product [168]. This flavin dependent, aldehyde-oxidizing protoluciferase produced sufficient light, and with an appropriate emission spectrum, to be detected by phototactic organisms. Ingestion by visually cueing animals of particles colonized and made luminous by these early luminous bacteria presumably enhanced their reproduction by bringing them into the animal’s nutrient-rich digestive system, ensuring the emitter’s survival and thereby possibly leading to selection for more intense light output [199]. It is possible that early evolutionary steps leading to protoluciferase involved oxygen detoxification activity that permitted early anaerobic organisms to survive an increasingly aerobic environment [120, 156]. An alternative hypothesis for the evolution of bacterial luciferase, involvement in DNA repair [32], has been refuted [194].

A single gene was hypothesized to encode bacterial protoluciferase [144]. Although a single-subunit protoluciferase, monomer or dimer, presumably would have differed somewhat from the modern-day luciferase  $\alpha$ -subunit and therefore

might have produced light, the inability of either of the extant  $\alpha$  or  $\beta$  subunits alone to produce light in vitro or in vivo [105] argues against the single-gene hypothesis. Alternatively, bacterial protoluminescence may have arisen following a gene duplication event that is postulated to have created *luxB* from *luxA* [10, 122, 144]. Based on amino acid sequence identities, a tandem duplication of the ancestral *luxA* gene, followed by sequence divergence in the duplicated gene, is thought to have given rise to *luxB*, leading to the formation of the heterodimeric luciferase present in extant luminous bacteria. Similarly, a tandem duplication of *luxB* followed by loss of approximately 300 nucleotides coding for N-terminus amino acids is thought to have given rise to *luxF* in a luminescent ancestor of *Photobacterium*; this gene apparently was later secondarily lost in the lineage giving rise to *P. leiognathi* [5, 10, 122, 144].

The association of the fatty-acid reductase genes, *luxCDE*, with *luxA* might have predated the *luxA* to *luxB* gene duplication event. Alternatively, the presence of ERIC sequences flanking *luxA* and *luxB* in *Ph. luminescens* [123] might mark an insertion of the *luxAB* genes into the fatty aldehyde reductase operon during the evolution of the bacterial luminescence system. The origins and evolution of other luminescence genes are not well understood [144]. The evolution of the bacterial luminescence system also involved recruitment of regulatory and other genes to the *lux* operon in some species (Fig. 2).

The energetic cost of light production, involving expenditures of carbon, nitrogen, and ATP in the synthesis of Lux proteins and in their enzymatic activities [41] indicates that luminescence has physiological or ecological importance for those bacteria able to express it. In the absence of selection to retain the *lux* genes, this cost would lead to loss of function through mutation and gene loss, an evolutionary scenario that probably accounts for the scattered incidence of luminous strains and species in *Vibrionaceae*. As outlined above, bacterial luminescence might have arisen evolutionarily as a means of coping with oxygen. Consistent with this possible function, luciferase, as an oxidase, might function as a secondary respiratory chain that is active when oxygen or iron levels are too low for the cytoplasmic membrane-associated, ATP-generating electron transport system to operate. This activity would allow cells expressing luciferase to reoxidize reduced coenzyme even when oxygen levels are low [75, 76, 78, 135]. Supporting this view, growth of cytochrome-deficient luminous bacteria is dependent on induction of luciferase, limitation for iron stimulates light production, low oxygen levels promote the luminescence of some luminous bacteria, and luciferase synthesis can be induced under anaerobic conditions [47, 82, 116, 118, 133]. As an alternative or supplement to the electron transport system, the activity of luciferase in reoxidizing reduced coenzyme could permit cells of luminous bacteria in low oxygen habitats, such as in animal gut tracts, to continue to transport and metabolize growth substrates, thereby continuing to gain energy through substrate-level phosphorylation. Furthermore and consistent with the ecological selection scenario above, light production presumably facilitates dissemination of luminous bacteria. The feeding of animals on luminous particles (decaying tissues, fecal pellets, and moribund animals infected by luminous bacteria), to which they are attracted,

would bring the bacteria into the animal's nutrient-rich gut tract for additional rounds of reproduction followed by dispersal [78, 135]. Recent evidence is supportive of this possibility [211]. Alternatively, the function of the bacterial *lux* system might be to generate a halotolerant flavodoxin, with light emission an incidental consequence [95]. Future studies might provide additional support for these and other proposed functions for luminescence, such as a physiological role for luciferase activity in bioluminescent symbioses with fish and squid. Proposed functions will be held to the standard of a demonstrated selective benefit for luminescence, either physiological or ecological.

## 7 Outlook

Substantial progress has been made in the past few years in defining the taxonomy and phylogenetic relationships of luminous bacteria and more fully characterizing the biochemistry and genetics of bacterial luminescence. Despite this progress, current understanding of the physiological function and ecological benefit of luminescence in bacteria remains limited. The long-standing question, "Why do bacteria make light?" remains essentially unanswered. A more detailed knowledge of the evolutionary origins and biochemical uniqueness of bacterial luciferase (e.g., [104]) and a more comprehensive understanding of the phylogenetic distribution of *lux* genes through whole-genome sequence analysis (e.g., [193]) in the context of the ecology of these bacteria (e.g., [211]) are likely to provide clues. Particularly insightful, however, will likely be detailed comparative physiological analysis of genetically defined mutants (e.g., [116]), an approach that addresses the core question and provides an experimental foundation for testing specific functional hypotheses.

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