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Illuminating Bacterial Communication

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Abstract

The scientific marvel of bioluminescence occurs when organisms are able to produce and emit light. This type of chemiluminescence is observed in several bacteria, fungi and mammals. Research on bacterial bioluminescence has illimunated much about the chemical regulation of this phenomenom and provides insight into how these natural displays of light might one day be employed in electricity-free lighting for human population. In this brief review, we will provide an overview of bioluminescence highlighting the impressive regulation of naturally occuring community-wide displays of bacterial light production.

Keywords: Bioluminescence, Quorum Sensing, Chemical Signaling, Vibrio harveyi

1.0 Introduction

Since the discovery of fire, humanity has been fascinated with the development of methods for the illumination of the environment we live in, adding to our safety, comfort and enabling innumerable societal advances. Our combined interest in having a regulated method for inducing a luminescence response has led to advances in many areas including electric transmission and lighting technology. These advances are readily apparent in night time images captured from space in which the outlines of human communities are defined in light (Figure 1B). While the luminescence output pales in comparison to the light levels from modern anthropogenic lighting, bacterial communities can, in certain conditions, produce luminescence outputs which are similarly visible to orbiting satellites (Figure 1A).

1.1 Bacterial Bioluminescence

Bioluminescent bacteria primarily inhabit the ocean, where they either live free or symbiotically. *Vibrio fischeri* (also known as *Allivibrio fischeri*) is a well studied symbiotic bioluminescent bacteria, notably interacting with *Euprymna scolopes* (Hawaiian bobtail squid). *Photobacterium leiognathi*, which inhabits apogonid, leiognathid, and morid fishes, is another example of bioluminescent bacteria that participate in symbiotic relationships.[2] The free-living bioluminescent bacteria *Vibrio harveyi* is believed to be a primary contributor to the "milky seas" effect (Figure 1B).[1] The regulation of bioluminescence expression in both *Vibrio fischeri* and *Vibrio harveyi* have been well investigated, occurring through a mechanism of chemical communication known as quorum sensing.[3-5] Known as *Allivibrio fischeri*) is a well studied symbiotic bioluminescent bacteria, notably interacting with *Euprymna scolopes* (Hawaiian bobtail squid). *Photobacterium leiognathi*, which inhabits apogonid, leiognathid, and morid fishes, is another example of bioluminescent bacteria that participate in symbiotic relationships.[2] The free-living bioluminescent bacteria *Vibrio harveyi* is believed to be a primary contributor to the "milky seas" effect (Figure 1B).[1] The regulation of bioluminescence expression in both *Vibrio fischeri* and *Vibrio harveyi* have been well investigated, occurring through a mechanism of chemical communication known as quorum sensing.[3-5]

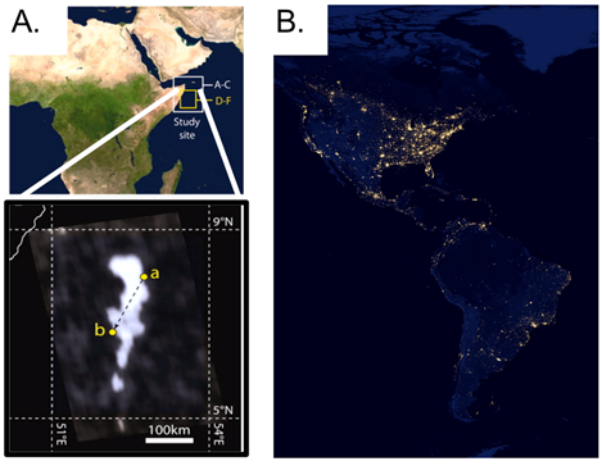

Figure 1. A. Bacterial bioluminescence in the open ocean off the coast of Africa.[1] Image Copyright (2005) National Academy of Sciences, U.S.A. **B.** Light production from North and South America. Image courtesy of NASA.

From a chemical perspective, bioluminescence occurs as the product of an oxidation reaction that releases energy in the form of emitted light. Enzymes known as luciferases catalyze the reaction on a class of substrates broadly

referred to as luciferins, ultimately producing an intermediate in an excited state that then decays to its ground state and emits light. In the case of bacterial luciferases, two molecules are oxidized concurrently by molecular oxygen, $FMNH_2$ (a reduced form of flavin mononucleotide, FMN) and a long-chain aldehyde. Along with the generation of FMN and the corresponding carboxylic acid, 0.1 molar equivalents of hν are produced, equating to a relatively large bioluminescence quantum yield of 10% according to the following equation.[3]

$$FMNH_2 + RCHO + O_2 \rightarrow FMN + RCOOH + H_2O + 0.1\ h\nu$$

All luciferases, that have been investigated, consist of a conserved heterodimer structure. The α and β subunits are functionally distinct. Binding of both substrates is known to occur on the α subunit, whereas the β subunit serves an unknown role, but is essential for luminescence. Specificity for $FMNH_2$ by luciferase is high, although some luminescence has been observed using other flavin derivatives. The other substrate also has some degree of specificity, as only aliphatic aldehydes with chains longer than eight carbons are active and the exact chain length has a significant effect on the reaction kinetics. In *Vibrio* sp. bacteria the bioluminescent output of this process occurs with an emission maxima of ~490 nm having a half-bandwidth of ~70 nm.[2]

The luciferin-luciferase system in these bacteria is encoded by a group of genes known as the Lux operon, which ultimately is regulated by quorum sensing. Five genes (luxCDABE) are recognized to be essential for light emission in *V. harveyi*.[3] The luciferase is coded for by the *lux* A and *lux* B genes, whereas the *lux* CDE genes produce the fatty acid reductase complex that creates the necessary aldehyde for the light-emitting reaction. Another gene known as *fre* (or *lux* G) is located next to *lux* E and codes for the $FMNH_2$ substrate. The luxCDABE genes are, in turn, regulated by the process of bacterial quorum sensing through a complex regulatory cascade involving several proteins and small RNA's.

Quorum sensing involves three fundamental steps: 1) the bacterial biosynthesis and release of specific chemical cues into the surrounding environment, 2) receptor recognition of the signal and 3) integration of the signal binding into changes in gene expression. The process of quorum sensing enables population density-dependent regulation of genes (Figure 2). At low bacterial cell density, while signaling molecules are being produced and released into the surrounding environment, the concentration of signaling molecule remains below the required threshold for receptor binding. As the population of bacteria increases to a high cell density state, the concentration of signaling molecule in surrounding environment has similarly increased. Once the concentration of quorum sensing signal in the surrounding environment has increased beyond a threshold level for receptor binding, the quorum sensing circuit is activated and changes in the expression level of quorum sensing-regulated genes, including the upregulation of bioluminescence in *V. harveyi*, occurs.

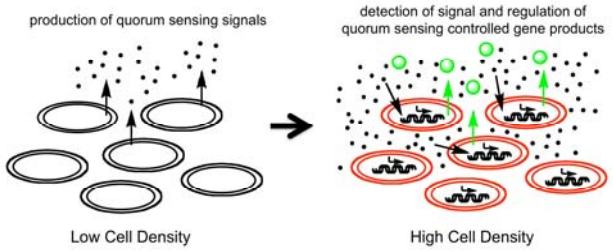

Figure 2. Overview of the process of bacterial quorum sensing enabling population-wide regulation of gene expression.

From a regulatory perspective, the human and bacterial luminescent displays visible from space (e.g. Figure 1) are considerably different and illustrate one of the remarkable features of the efficiency of the chemical regulatory pathway operative in the bacterial regulation of this process. While the two processes of luminescent output are quite distinct in most ways, they are both expressions of a population-wide response to a signaling cue. In the anthropogenic production of light, the process largely reflects a community-wide response to an external signaling cue, the onset of darkness. This includes automatic lights, such as street lights, which are regulated by a darkness sensor and therefore respond to the same signaling input. In contrast to this, organization of the bacterial production of a luminescence response also reflects a community-wide response however to an internal signaling cue, specifically the chemical signaling molecule(s) produced by the bacteria involved and integrated into a response through quorum sensing. The unified, community-wide response of a population to a given signal requires, in the simplest sense, recognition of the signal cue and execution of a defined response to the signal. The efficiency of corresponding a signal input to an output has been popularized in audio recordings, where it is referred to as signal fidelity. In community-wide behaviors, signaling fidelity can therefore be defined as the efficiency by which a signal cue is transformed into a response. Therefore, for much of anthropogenic light production the signaling cue represents a unified response to an external signal. Coordinating the response of a population to an external, environmental cue can be viewed as one of the simplest types of regulatory processes and in these cases the responses are largely innate responses, which positively impact the survival of individuals within the population. In the production of anthropogenic light, the world population of $\sim 7 \times 10^9$ efficiently act as a single coordinated population in their response to the environmental cue of darkness to up-

regulate light production taking advantage of the many developments in electric transmission and lighting to achieve this result. Contrasting this is the bacterial regulation of light production to an internal cue. The bacterial process of quorum sensing, which controls bioluminescence in *Vibrio* sp. bacteria, up-regulates light production only in response to a specific chemical signaling cue, which is synthesized and released into the environment by the bacteria. The amazing signaling fidelity of this chemical cue is manifested in the image of bioluminescence presented in Figure 1B. This image represents the coordinated response of a population of ~$4x10^{22}$ individual bacteria. This one luminescence display represents the unified action of a population of bacteria 13 orders of magnitude larger then the entire human world population.

1.2 Regulation of Bacterial Bioluminescence

In the marine bacteria *V. harveyi,* this impressive regulatory process occurs through the quorum sensing utilizing three separate chemical signals (Figure 3).[4,6,7] At low cell density, the signal molecule synthases LuxM, LuxS and CqsA produce the quorum sensing signals, (*S*)-3-hydroxy-*N*-((*S*)-2-oxotetrahydrofuran-3-yl)butanamide (3-hydroxy-C4-HSL, green), 4,5-dihydroxy-2,3-pentanedione (in seawater exists as the borate ester AI-2, blue) and (*Z*)-3-aminoundec-2-en-4-one (Ea-C8-CAI1, red), respectively. At low cell density, these chemical signals are not detected by their corresponding dimeric transmembrane histidine kinase receptors. Each of these receptors is selective for the detection of a single chemical cue. The LuxN receptor binds the homoserine lactone (HSL) ligand, 3-hydroxy-C4-HSL (green) the LuxPQ receptor complex responds to the borate ester AI-2 (blue) and the CqsS receptor is sensitive to Ea-C8-CAI1 (red). In the absence of signal molecule, these receptors function as kinases phosphorylating down-stream response regulator proteins in an ATP-dependent process. The resulting phosphorylation cascade ultimately leads to the phosphorylation of the response regulator LuxO which activates the transcription of five small regulatory RNA's (Qrr sRNA's). The sRNA's, together with the RNA chaperone protein Hfq, inhibit the production of LuxR and thereby the bioluminescence genes are not expressed. Once the concentration of the quorum sensing signals increases above the threshold level for detection by the quorum sensing receptor proteins, their activity changes from kinase to phosphatase. This event, induced by ligand binding, reverses the flow of phosphate through the system and leads produced. In the absence of Qrr sRNA's, LuxR is produced and transcriptionally upregulates the luxCDABE genes, in addition to numerous other changes in gene expression, inducing bacterial bioluminescence.

2.0 CONCLUSION

Since the discovery of quorum sensing regulation of bioluminescence in *V. harveyi* researchers have illuminated the details of the regulatory circuit that enables these bacteria to "turn on the lights" in a carefully controlled, community-wide manner. Beyond the regulation of bioluminescence, quorum sensing has been identified to play a central role in numerous collective behaviors including symbiosis, plasmid conjugation, competence, virulence factor production, and biofilm formation.[8,9] Importantly, quorum sensing plays a major role in bacterial gene regulation in a wide variety of environments and there is convincing data showing that bacterial pathogens rely on these communication systems to establish and sustain infection.[10,11] Accordingly, an ongoing aspect of study is research directed at the development of anti-quorum sensing therapies for use as anti-virulence alternatives to traditional antibiotics or as supplements to existing treatments.[12-17] The future for research in the field of bacterial quorum sensing remains bright with many new applications and discoveries expected. Developing technology to harness bioluminescence for the illumination of our human habitats will require innovations in several scientific areas, bridging research in materials science, luminescence studies and biotechnology; however projects in this area are already being deployed.[18-20]

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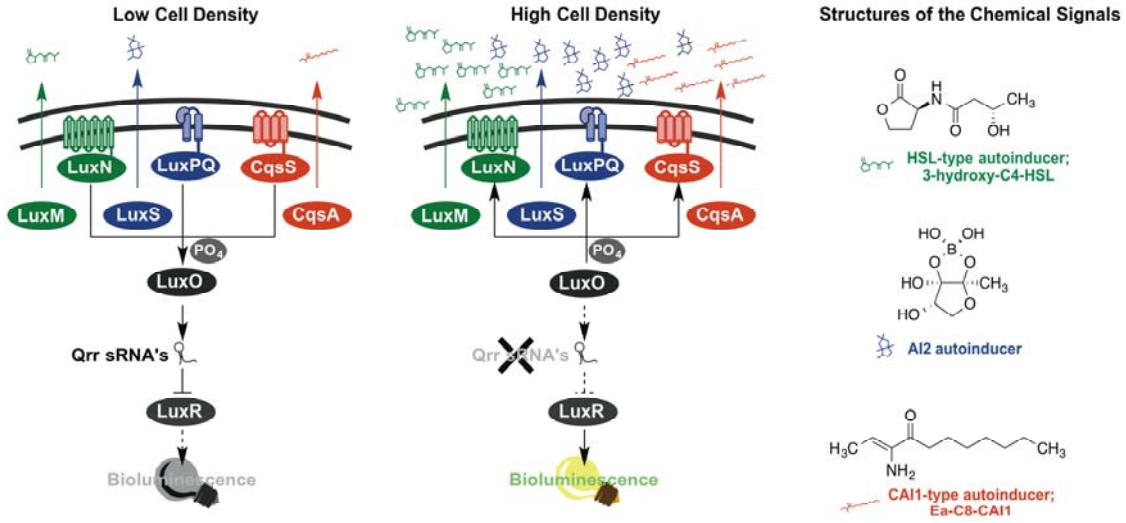

Figure 3. Simplified diagram of the chemical signaling pathway operative in *V. harveyi* quorum sensing.