Bioluminescence

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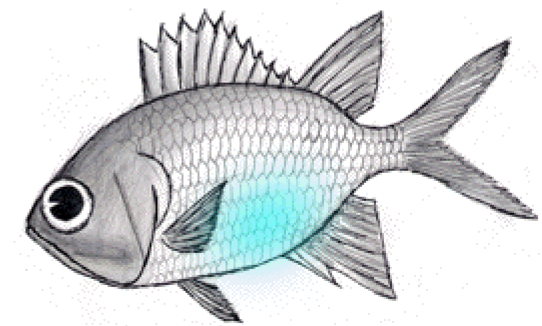
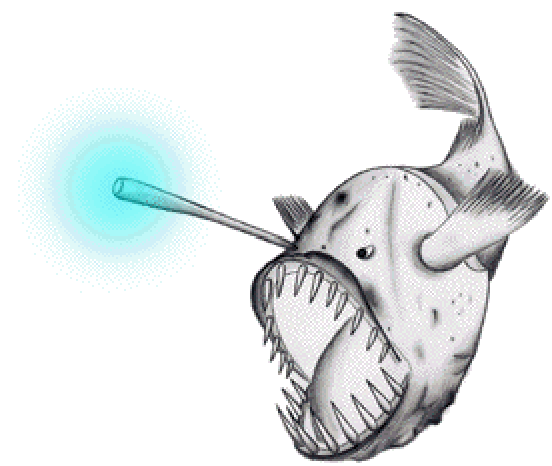
Information Source: http://photobiology.info/Lin.html

Bioluminescence

- **Bioluminescence** is an emission of cold light by enzyme driven reaction within certain living organisms.
- The most abundant and widely distributed light emitting organisms are luminescent bacteria.
- Such organisms are either found as free-living in the ocean or in symbiotic relationship with the marine host.
- In symbiosis, the bacteria are nourished with readily available food sources for growth, and at the same time the host utilizes the adopted illumination to communicate, to attract prey, and to masquerade itself from predators.



Pinecone fish utilize luminous bacteria, colonized in the ventral cavity



The deep sea Angler fish carries luminous bacteria in a light emitting rod, which attracts prey to the front of its mouth.

... Bioluminescence

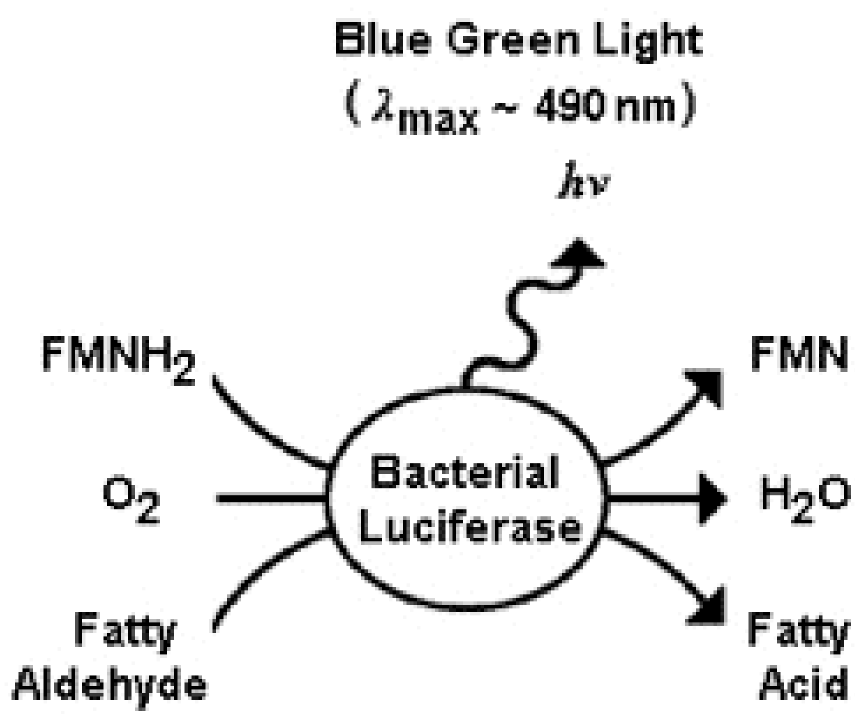
- There are three major genera, into which most luminous bacteria are classified; *Photobacterium, Vibrio*, and *Photorhabdus*.
- Species existing in the marine environment are mainly categorized into the *Photobacterium* and *Vibrio* genera, and the terrestrial species are classified into the *Photorhabdus* (previously designated as *Xenorhabdus*) genus.
- Species within the *Photobacterium* genus are generally light organ symbionts of marine animals, whereas the *Vibrio* species exist as free-living forms as well as symbionts in the sea.
- Many luminous bacteria are parasitic, with *Photobacterium* and *Vibrio* families infecting marine crustacea, and *Photorhabdus luminescens* infecting terrestrial insects, such as caterpillars, with nematodes as the intermediate host for the bacteria.
- In addition, free-living luminous bacteria that are dispersed in the seawater can often be found in both the gut tract and on the skin surface of almost all marine animals as non-specific parasites.

Biochemistry of the Bacterial Bioluminescence Reaction

- Bacterial luciferase is the enzyme that catalyzes light emission.
- However, the catalytic machinery also involved the enzymes that supply and regenerate the substrates of bacterial luciferase.
- The DNA sequences coding the proteins in the luminescent system are termed the lux genes.
- Bacterial luciferase is a heterodimer, composed of two different polypeptides, designated alpha and beta encoded by the *lux*A and *lux*B genes, respectively.
- The active site is located within the α subunit.
- The substrates of bacterial luciferase are reduced flavin mononucleotide (FMNH), molecular oxygen, and long chain fatty aldehyde.
- The excess energy, which is liberated from the oxidation of $FMNH_2$ and aldehyde concomitant with the reduction of molecular oxygen, is released as blue/green light emission (MAX ~ 490 nm).

The net chemical equation of the bacterial luciferase catalyzed reaction

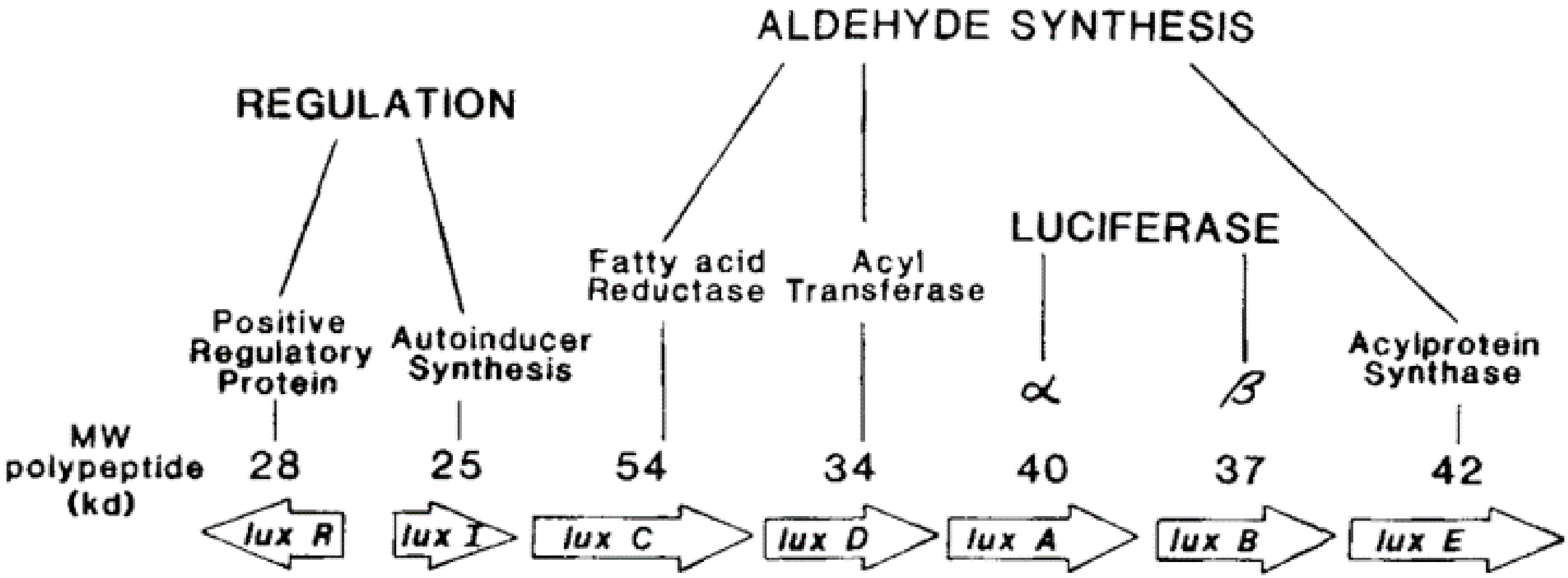
- The characteristic color indicates the energy level of the photon that was produced when the excited electron on the flavin chromophore returns to the ground state.
- Flavin analogs with substituted atoms in the chromophore moiety resulted in different luciferase emission colors.



lux operon

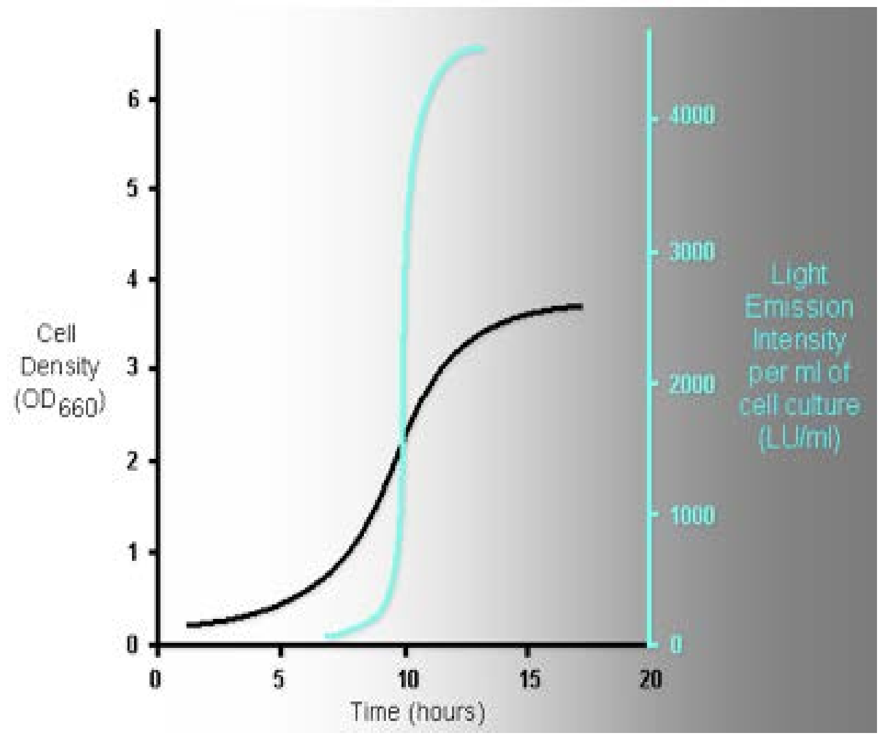
- In order for light emission to occur for prolonged period of times, substrates must be supplied continuously to bacterial luciferase.
- The constant light emission in luminous bacteria must therefore be maintained by several different enzymes continuously generating the substrates for the bioluminescence reaction.
- Those enzymes that replenish the aldehyde substrate are coded on the *lux* operon; in particular, the fatty acid reductase, a multienzyme complex, whose lux genes (*luxC, luxD*, and *luxE*) immediately flank the *lux*A and *lux*B genes of luciferase.
- Other genes including *lux*F, *lux*G, and *lux*H, whose functions are neither clearly defined nor apparently necessary for bioluminescence are also found in some *lux* operons.

lux Operon


ALDEHYDE SYNTHESIS
REGULATION
LUCIFERASE
Fatty acid Reductase
Acyl Transferase
Positive Regulatory Protein
Autoinducer Synthesis
α
β
Acylprotein Synthase
MW polypeptide (kd)
28
25
54
34
40
37
42
lux R
lux I
lux C
lux D
lux A
lux B
lux E

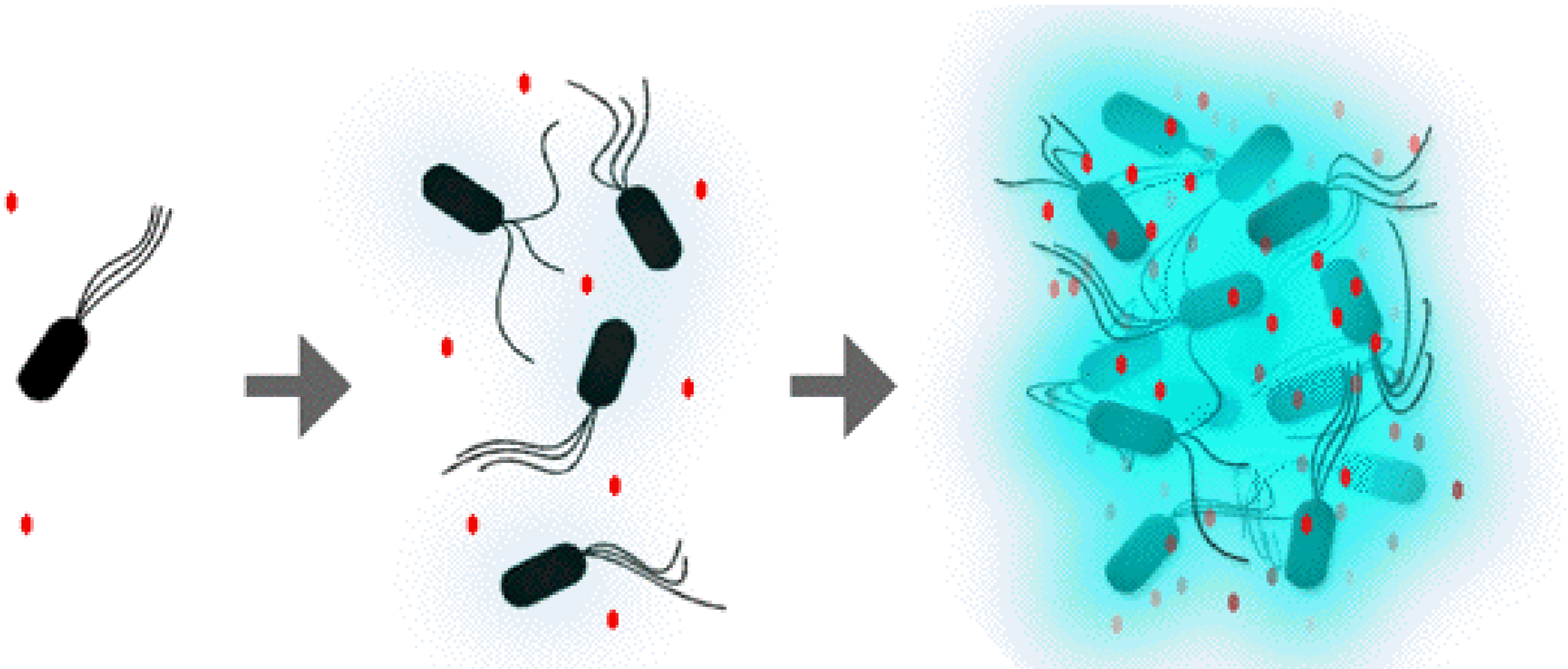
Regulation of the *lux*CDABE Expression in Luminous Bacteria - Quorum Sensing

- In the laboratory set-up, luminous bacteria growing in liquid media at low cell density give off a minimal amount of light, because of the dormancy of the expression of *lux*CDABE genes and the deficiency in the level of substrate for the bacterial luciferase reaction.
- From the mid to the end of the exponential growth period, the intensity of the light emission rises dramatically as the result of the rapid accumulation of the synthesized substrates and enzymes from the activation of the expression of the *lux*CDABE genes.



Quorum Sensing & Bioluminescence

- In bacterial bioluminescence the catalysts that induce the expression of the *lux*CDABE gene are regulatory proteins, and a small chemical compound, which is commonly called the autoinducer.
- The autoinducer is a small metabolic product, which freely diffuses across the cellular membrane, and is excreted to the extracellular environment during the early stage of cell growth.
- It is known that marine luminescent bacteria that live free in the ocean do not emit light, while bacteria living as symbionts in marine organisms and/or in a localized, confined environment emit a high level of light.
- Therefore, in order for the light emission to occur, luminous bacteria have to grow in a confined, nutrition-rich environment, in which the autoinducer can be accumulated.
- When the concentration of the autoinducer increases to a certain level in the extracellular environment, the autoinducers will activate the luminous system of luminous bacteria.
- This process has been called "quorum sensing", as the bacteria literally sense when a sufficient concentration of bacteria has been reached based on the accumulation of autoinducer.



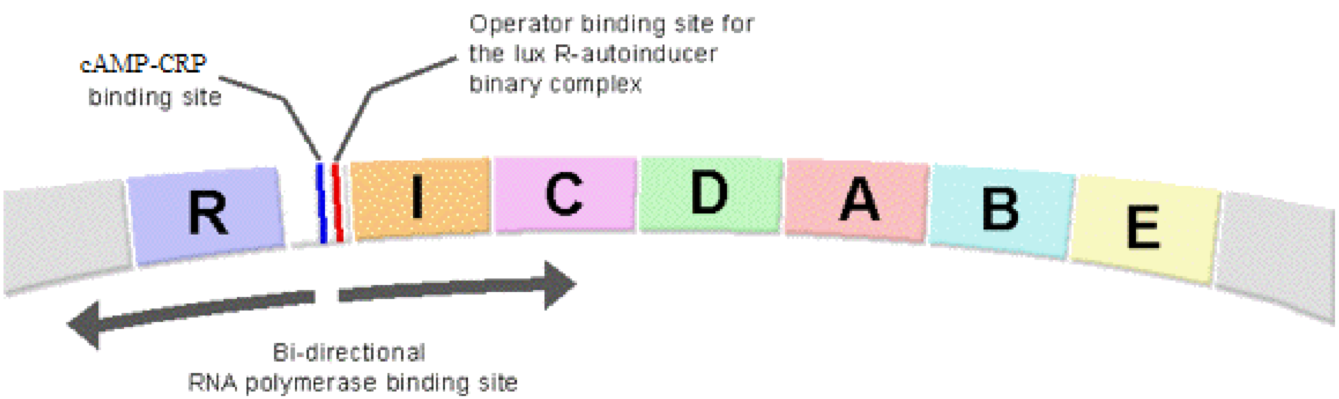
At low cell density the autoinducers (red dots) that are produced diffuse through the cell membrane into the growth media.

As the cell growth continues, the level of autoinducers in the media will start to accumulate in a confined environment. A very low intensity of light emission can be detected.

High levels of autoinducers activate the luminescent systems of the luminous bacteria in the media. The regulatory response to the autoinducer leading to induction of expression of the *lux*CDABE genes is termed "QUORUM SENSING."

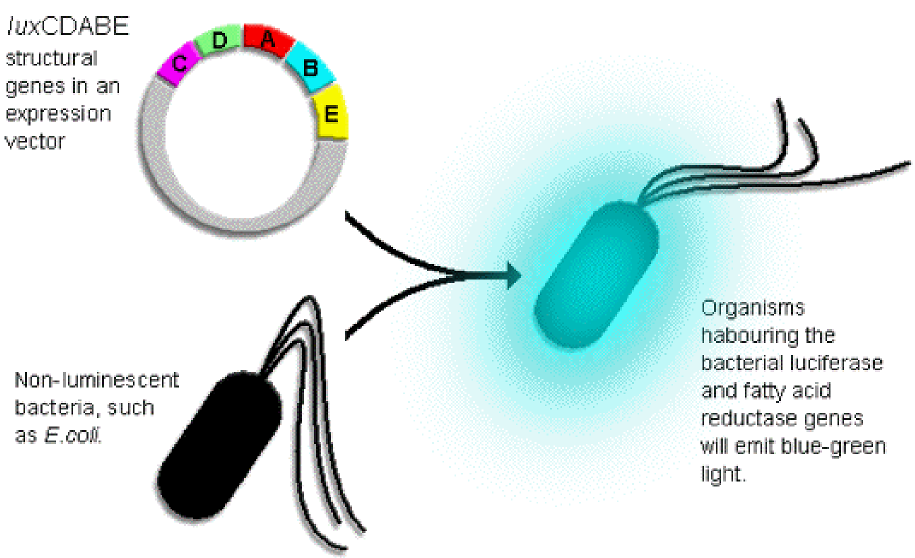
The Autocatalytic Mechanism Involved in the Regulation of *V. fischeri lux*CDABE Expression

- The autoinducer that turns on the bioluminescent system of *V. fischeri* is 3-oxohexanoyl-homoserine lactone, synthesized by the autoinducer synthetase coded by the *lux*I gene.
- When a critical concentration of 3-oxohexanoyl-homoserine lactone is established in the extracellular environment, autoinducer starts to accumulate inside the luminous bacteria and interacts with the regulatory protein coded by *lux*R.
- Binding of the activation complex (autoinducer-LuxR) to the operator site in the regulatory region between the *lux*R and *lux*ICDABE operons along with a cAMP-CRP (cAMP-cAMP receptor protein) complex at a CRP binding site, leads to RNA polymerase being recruited to the bi-directional promoter site to transcribe the downstream lux genes.



The Utilization of Bacterial Bioluminescence as a Biosensor and Reporter of Gene Expression

- The "insertion" of the foreign *lux*CDABE structural genes into the organism allows the fatty acid reductase enzyme complex and luciferase to be expressed, and the function of luciferase confers the organism the ability to emit light.
- The adaptation of light as an accessory feature of normally nonluminescent bacteria has provided researchers an easy alternative to measure and detect the growth and living conditions of bacteria.
- Example, bacterial bioluminescence serves as an indicator of bacterial growth allowing the proper dosages of antibiotics to be determined and effective treatment to be established.



Questions

- What is bioluminescence? Explain the biochemistry of bacterial bioluminescence.
- Write short note on *lux* operon and its reguation.
- How bioluminescence is linked with quorum sensing phenomenon?
- Write an essay on bioluminescence explaining the biochemistry of function and role of *lux* operon.