**Bacterial Bioluminescence**

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**Bioluminescence** is the biochemical emission of visible light by living organisms. As such, it is a special category of **chemiluminescence**. (Do not confuse bioluminescence with **phosphorescence**, which is a special form of **fluorescence**.)

The few known bioluminescent organisms are scattered among diverse taxa in 2 of the 3 domains. (There are currently no reports of bioluminescence among the Archaea). Apparently bioluminescence has evolved independently at least several dozen times in lineages as diverse as bacteria, fungi, dinoflagellates, insects, fish, and various other invertebrate phyla. Additionally, some nematodes, squid, and several groups of fish have evolved symbiotic relationships with bioluminescent bacteria. Hastings1 summarizes the phylogenetic distribution of symbiotic and non-symbiotic bioluminescent organisms.

The preeminent bioluminescent Bacteria are Gram-negative marine bacteria in the genera *Vibrio* and *Photobacterium*. Bioluminescence has also been observed in some strains in the bacterial genera *Alteromonas* and *Xenorhabdus*. These genera are all fairly closely related to each other, as are the biochemical systems responsible for light production. This may reflect a single evolutionary origin of bioluminescence in Gram-negative Bacteria, followed by horizontal gene transfer.
Biochemical Mechanism

Enzymes that catalyze light-emitting reactions in bioluminescent systems are called "luciferases". The organic co-substates for the luciferases are called "luciferins". Despite considerable diversity in luciferins (consistent with independent evolution of bioluminescent systems) all luciferases are flavin-dependent monooxygenase enzymes.

Flavin-dependent monooxygenase enzymes are widespread. They carry out oxidations of a wide array of organic substrates using molecular oxygen as electron acceptor, and a flavin coenzyme (such as FMN) as a secondary electron donor. Flavin-dependent monoxygenase activities often parallel the activities of more typical NAD-dependent dehydrogenases.

Most flavin-dependent monoxygenases are not involved in bioluminescence systems, but many of them do produce small amounts of light as a by product of their reactions. The light emission is actually due to an excited state of the flavin produced during the reaction cycle.

Bacterial luciferase is a heterodimeric flavin-dependent monooxygenase that catalyzes the oxidation of the 12-carbon aliphatic aldehyde, dodecanal (bacterial luciferin). The reaction mechanism, interestingly, is not very unique, being similar to that of other flavin-dependent monoxygenase enzymes.

Let’s back up to consider the global context of this reaction in biochemical evolution.

Typical aldehyde oxidations involve NAD-linked dehydrogenase reactions in which O₂ is not a substrate. The 2 electrons released by the oxidation of the aldehyde are transferred to NAD⁺, while the oxygen atom added to form the carboxyl group is derived from water.

"Typical" Aldehyde Oxidation by NAD-dependent Dehydrogenase
The corresponding reaction by a flavin-linked monooxygenase is:

\[
2e^- + \text{FMNH}_2 + O_2 \xrightarrow{\text{oxygenase}} O \text{R-C-OH} + \text{FMN} + H_2O
\]

In this case the oxygen added to the substrate comes from \( O_2 \), and water is a product, not a reactant. 2 of the 4 electrons needed to reduce the \( O_2 \) are derived from the aldehyde oxidation, while the other two are donated by the flavin. Note that in the dehydrogenase reaction \( \text{NAD}^+ \) is electron acceptor. In the case of the oxygenase, \( \text{FMNH}_2 \) is electron donor. The \( \text{NAD}^- \)-linked dehydrogenases are probably more ancient than the flavin-linked monooxygenases because they could have functioned on the anaerobic earth.

The significance of this is that the light emitting molecule is an excited form of the FMN produced as an intermediate in the reaction (NOT the dodecanal). Many flavin-dependent enzyme reactions emit light as a by-product; the bacterial luciferase is unusual only in that light production is highly efficient.

The high energetic cost of bioluminescence is incurred by the necessity of reducing the dodecanoic acid product back to dodecanal, and by the drain of electrons from \( \text{FMNH}_2 \) that might otherwise be used to generate ATP through electron transport.

All bacterial luciferases emit blue light, the color which is least absorbed in sea water. However, bioluminescence of some bacterial strains is yellow or green due to the presence of a fluorescent secondary protein.
**Genetics and Regulation**

Genes for luciferase and associated genes required for light production, are clustered in an operon called the *lux* operon.

![Diagram of Lux operon](image)

**Lux**  | **AHL (autoinducer synthase) mechanism not established**  
--- | ---  
A | Luciferase alpha subunit  
B | Luciferase beta subunit  
C | Fatty Acid Reductase Complex (reductase)  
D | Fatty Acid Reductase Complex (acyetyltransferase)  
E | Fatty Acid Reductase Complex (acyl protein synthase)  
G | function unknown – not required for bioluminescence

Expression of the lux operon is regulated by a type of control system referred to as "quorum sensing" which ensures that bioluminescence only occurs at high population densities. Light production is energetically expensive (see above). Small populations of bacteria would not produce enough light to be visible to macrofauna. This is consistent with the hypothesis that bioluminescence facilitates ingestion of heterotrophic bacteria by fish and other marine scavengers. (NOTE: Quorum sensing is referred to as "autoinduction" in some older literature.)

Quorum sensing was first discovered in the context of regulating bioluminescence in *Photobacterium* and *Vibrio* and was originally called "autoinduction". Quorum sensing has subsequently been described in many Gram-negative bacteria where it is used to regulate diverse physiological systems including toxin production in some pathogenins.

The species specificity of quorum sensing is due to differences in chemical structure of an acetylated homoserine lactone (AHL) which is referred to as the "autoinducer".

The AHL autoinducer works in concert with a regulatory protein which is an allosteric transcriptional activator. One of the genes activated by the regulatory protein codes for
an enzyme required for AHL synthesis, so that there is a positive-feedback control loop established. Note that this strategy requires a basal level of transcription and AHL synthesis in single isolated cells.

Expression is also regulated by catabolite repression (see Sec. 8.7).
Symbiosis

Some bioluminescent bacteria are symbionts (facultative or obligate) of marine fish or squid.

In most fish-bacterium symbioses (10 families of 5 orders), the bioluminescent bacteria are found in some part of the gut and are similar to free living, culturable, Vibrio or Photobacterium species.

Light organ symbioses in Anglerfish and Flashlight fish are obligate; the bacteria have not been cultured outside their hosts. PCR-based 16SrRNA sequencing of the light organ symbionts show they are pure cultures of a single symbiont strain derived from the same common ancestor as the facultative Photobacterium symbionts and free-living marine Vibrios. In 9 of 11 families females have luminescent lure; deep-sea predators; large suborbital light organs for conspecific communication.

The symbiosis between the squid Euprymna scolopes and Vibrio fisheri has been studied extensively as a model system for host-symbiont interactions and coevolution. Strains of V. fisheri are host specific. Molecular phylogeny of the host is congruent with that of symbiont. Young squid are specifically colonized by the appropriate strain of V. fisheri among the diverse strains present in seawater. The colonization of the light organ serves as a model for bacterial-host interactions.
Applications

Bacterial *lux* genes have been cloned and used as "reporter genes". Light emission can be easily detected and measured by simple electronic luminometers.

Bioluminescent bacteria are the basis of a general toxicity assay based on metabolic inhibition. Recognized by Std. Methods (1995) as BBT 8050. Instrumentation and reagents are marketed commercially by as the "Microtox" system by Microbics Corp., Carlsbad, CA.

The luminous organ of a marine fish is used by at least one cultural group as a lure for fish.
QUESTIONS

What features of bacterial bioluminescence suggest that light production itself (rather than oxygen removal or carbon metabolism) is the functional basis of the system?

What are the general biochemical properties of the bioluminescent system in bacteria?

What is quorum sensing and how does it operate in bacterial bioluminescence? Give one other example of quorum sensing involving an AHL autoinducer.

Define and distinguish the following terms:

- Bioluminescence
- Chemiluminescence
- Fluorescence
- Phosphorescence

How could quorum sensing be useful to a pathogenic bacterium?
1 Hastings, J. W. 1983

2 Haygood, M. G. and Distel, D. L. (1993)
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3 Pennisi, E. (1996)
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5 Engebrecht, J. et al. (1985)
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Sec. 8050, p. 8-32.

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