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GLOWING GENES: THAT LIGHT THE NIGHT

Kirti Dahigaonkar*, Qamrunnisa Abdul Wahid Shaikh, Heena Riyaz Sayyed, Sana Firoz Bagwan, Rais Imrooz Mohammed Sikander Kasar, Pratibha Jadhav, Jaspal Kaur Oberoi

Abeda Inamdar Senior College, Department of Microbiology.

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***Corresponding Author**
Kirti Dahigaonkar
Abeda Inamdar Senior College, Department Of Microbiology.

ABSTRACT

Can plant glow and replace other sources of light? Yes plants can! by using bioluminescence genes. Bioluminescence is the production and emission of light by a living organism. It occurs widely in marine vertebrates and invertebrates as well as in some fungi, including some bioluminescent microbes and terrestrial invertebrates such as fireflies. Bioluminescence or.ganisms uses luciferase enzyme.

$$\text{Luciferin} + O_2 \xrightarrow{\text{Luciferase}} \text{oxyluciferase} + \text{light}$$

There are different sources of luciferase enzyme such as fireflies luciferase(FLUC), sea pansy luciferase (RLUC), bacterial luciferase(*lux*AB), click beetle luciferase(CbLUC). Various plant species in which luciferase reporters have been successfully utilized: *Arabidopsis thaliana* (FLUC and RLUC genes), *Nicotianatabacum* (FLUC genes), *Daucuscarota (lux*AB genes). Traditional modes of electric lighting can be a living source of biological light which reduces the ecological impact of lighting. Which represent 19% of global electric consumption and 5% of CO_2emission, visual pollution and luminous pollution. It is not to entirely replace electric light with bioluminescence but to use it as an alternative solution able to reinvent uses of light. From the above discussion we can conclude that bioluminescent organisms can be inserted in plants and can be used as a source of light. Our goal is to change the way we produce and use light. We want to offer a global solution that will reduced the 19% of electricity consumption used to produced light.

KEYWORDS: *lux* and *luc* receptor genes, light emission, bioluminescent microbes, gene expression, luciferase enzyme, transgenic plant.

INTRODUCTION

Electricity is an invisible and naturally occurring force that can be seen in natural phenomena as lightening. The utilization of electricity offers numerous conveniences but it can also harm the environment. Effects of electricity: green house gases emission, pollution, acid rain and injuries to wildlife. Can plants glow and replace other sources of light? Yes plants can! By using bioluminescent genes.

- Bio-living matter
- Lumin-to fill with light
- Escence- process that changes state.

Nature is beautiful, there is wide variety of life on earth. Genes from bioluminescent organisms are used as innovative tool for the revolution of environment. Bioluminescent genes have been transferred from various sources as firefly, sea pansy, click beetle and bacteria to makes the plants luminescent. The main aim is to transfer electric source of light into biological source of light using Bioluminescence. It is not to entirely replace electric light but to use it as an alternative.

BIOLUMINESCENCE

Production and emission of light by a living organism is called as a Bioluminescence. Bioluminescence is the conversion of chemical energy into light within a living organisms. In marine vertebrates and invertebrates, as well as in microorganisms including some bioluminescent bacteria, some fungi, and terrestrial invertebrates such as fireflies, coelenterate etc bioluminescence widely occurs. In some animals, the light is produced by symbiotic organisms such as *Vibrio* bacteria. The enzyme that catalyze the bioluminescence reaction in the organisms are called luciferase, and in most cases the substrate are designated as luciferin.[1] This property was initially recognized more than three centuries ago by Robert Boyle, who demonstrated that air (0_2) was required for light emission by rotting wood containing luminescent fungi or bacteria.[2] A little more than two centuries later, Dubois demonstrated, by performing classic in vitro experiments with hot- and cold-water extracts from the luminous fire beetle and the boring clam, the requirements for luciferase, luciferin(s), and oxygen in these luminescence systems.[3] Most marine bioluminescent organisms emit light in the range of 440-480nm.

BIOLUMINESCENT BACTERIA

Bioluminescent organisms are widely distributed in nature and comprise a remarkably diverse set of species.[4,5,6,7,8] Among the light-emitting species are bacteria, dinoflagellates, fungi, fish, insects, shrimp, and squid. This set of organisms includes terrestrial, freshwater, and marine species from almost 50% of the different phyla in the animal and plant kingdoms.[9] Luminous bacteria are the most abundant and widely distributed of the light-emitting organisms and are found in marine, freshwater, and terrestrial environments. The most common habitats for these bioluminescence bacteria are as free-living species in the ocean, in symbiotic relationship as gut symbionts in the digestive tracts of marine fish,in parasitic relationship as parasites in crustacean and insects , as saprophytes growing on dead fish or meat, and as light organ symbionts in the fishes and squid.[6] These bacteria are all gram-negative motile rods and can function as facultative anaerobes.[10,11]

Almost all luminous bacteria have been classified into the three genera *Vibrio, Photobacterium,* and *Xenorhabdus,* with most of the species being marine in nature.[4,11] Only *Xenorhabdus* species infect terrestrial organisms.[12] *Vibrio harveyi, V. fischeri, Photobacterium phosphoreum, P. leiognathi,* and *Xenorhabdus luminescens* are light-emitting bacteria that have been investigated in most detail. Because there are many different isolates and sources of each species, significant differences may exist between the *lux* systems from luminescent strains of the same species. Other species of luminescent bacteria are *V. logei, V.splendidus, V. cholera,*[13] a freshwater species, and one species (*Altermonashaneda*) classified in the genus *Alteromonas.*[14] Cloning and expression of the DNA coding for luciferases from different luminescent organisms, including marine and terrestrial bacteria[15,16,17,18,19,20,21,22,23] fireflies and click beetles,[24,25] and the jellyfish (Aequorea spp.)[26,27] and the crustacean ostracod (*Vargula* spp.)[28]

Some bioluminescence bacteria are listed below in details:

I. Vibrio harveyi

Vibrio harveyi is a Gram-negative, bioluminescent, marine bacterium in the genus *Vibrio*. *V. harveyi* is rod-shaped, motile (via polar flagella), facultatively anaerobic, halophilic. It does not grow below 4 °C or above 35 °C. *Vibrio harveyi* can be found free-swimming in tropical marine waters, as both a primary and opportunistic pathogen of marine animals, including oysters, prawns, lobsters, the common snook, barramundi, turbot, milkfish, and seahorses and commensally in the gut microflora of marine animals .[29] based on samples taken by ocean-

going ships, it is thought that cause of the milky seas effect, in which, during the night, a uniform blue glow is emitted from the seawater is because of bioluminescent bacteria *V. harveyi* . Some glows can cover nearly 6,000 sq mi (16,000 km^2).[30]

II. *Vibrio fischeri*

Vibrio fischeri is a Gram-negative, rod-shaped bacterium found globally in marine environments.[31] V. fischeri has bioluminescent properties, and is found predominantly in symbiosis with various marine animals, such as the Hawaiian bobtail squid. It is heterotrophic, oxidase-positive, and motile by means of a single polar flagella,[32] composed of a cell wall that consists of an outer membrane containing lipopolysaccharides, a periplasmic space with a peptidoglycan layer, and an inner, cytoplasmic membrane.[33] Free-living *Vibrio fischeri* cells survive on decaying organic matter. *Vibrio fischeri* having the *lux* operon is responsible for the production of bioluminescence.

III. Photobacterium phosphoreum

Photobacterium phosphoreum was first isolated from the aquatic environment in the late 1880's by the Dutch microbiologist Martinus Beijerinck (1851-1931).[34] It is a Gammaproteobacteria which are Gram-negative, usually motile rods, are mesophilic and chemoorganotrophiccan grow in anaerobic conditions, and emits a blue-green light.[34] *Photobacterium phosphoreum* is one of many organisms that produce bioluminescence in marine organisms. *P. phosphoreum* is a light organ symbiont, living in the gut of the fish where metabolites are provided in exchange for bioluminescence, which is used for communication, prey attraction, and predator avoidance.[34] *P. phosphoreum* is psychrotolerant and often thrives in low temperatures but can be inhibited at temperatures above 25 degrees Celsius.[35,36] Bioluminescence has been strongly linked to cell density, and bacteria living freely in the ocean are not bioluminescent as they are in the light organs of the host organism.[37] The factors that makes *P.phosphoreum.*

Reliable reporter for assessing toxicants like heavy metals, alkanes, hydrocarbons, pesticides etc present in aquatic samples are the cost performance of bioluminescence, responsibility and simple operations. Due to its reliability it is being used for measuring the toxicity in aquatic environments caused due to biodegradable water toxins. *P.phosphoreum* play an important role in packed chilled fillets of fish as spoilage bacterium as it enhances the growth in packed products of fishes and causing the spoilage and fishy flavor.[38]

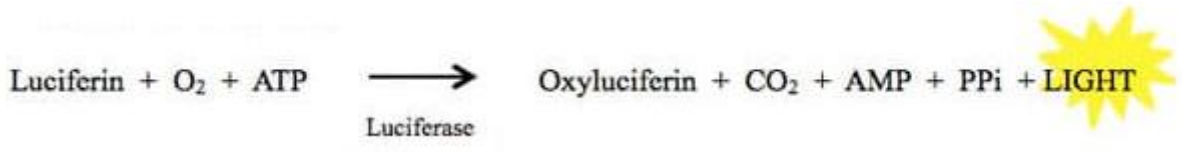
IV. Photorhabdus luminescens /Xenorhabdus luminescens

The lethal pathogen of insects which are mostly found in the gut of an Entomopathogenic nematodes of the family Heterorhabditidae are nothing but the *Photorhabdus luminescens* which was previously known as *Xenorhabdus luminescens*. When the nematode infects an insect, *P. luminescens* is injected into the blood stream of insect and by producing toxins such as the high molecular weight insecticidal protein complex Tca it rapidly kills the insect (within 48 hours).[39] *P. luminescens* also produces a proteic toxin through the expression of a single gene called *makes caterpillars floppy* (mcf).[40] nematode and bacteria gain nutrients by secreting enzymes. These enzymes break down the body of the infected insect and convert it into nutrients . In this way, both organisms nematode and bacteria gain enough nutrients to replicate (or reproduce in the case of the nematode) several times. When nematode reproduced the bacteria enter the nematode progeny. It has been reported that infection by this bacterium of the wounds of soldiers in the American Civil War caused the wounds to glow, and that this aided the survival of the soldiers due to the production of antibiotics by *Photorhabdus luminescens*.[41,42] This led to the phenomenon's nickname "Angel's Glow."[43] It is a source for bioluminescence imaging.

LUCIFERASE AS REPORTER GENE ACTIVITY IN PLANTS: Light produced by a chemical reaction within an organism is called as bioluminescence. At least two chemicals are required: 1) Luciferin 2) Luciferase

“Luciferin” produces light and the “luciferase” catalyzes the reaction. Most bioluminescent reactions involve luciferin and luciferase. Some reactions, however, do not involve an enzyme (luciferase). These reactions involve a chemical called a photoprotein. Photoproteins combine with luciferins and oxygen to produce light, but it required another agent, often an ion of the element calcium to produce the light. For the light-emitting compound found in organisms that generate bioluminescence is term as Luciferin (from the Latin lucifer, "light-bringer"). Luciferins typically undergo an oxidation with the help of enzyme i.e luciferase enzyme and the resulting intermediate emits light upon degrading to its ground state. Luciferin is a light producing substance and luciferase is an enzyme that catalyzes the reaction. The luciferase is a protein known as the photoprotein, and the light making process requires a charged ion to activate the reaction.Coelenterazine is the most "popular" of the marine luciferins, found in a variety of phyla. This molecule can occur in luciferin-luciferase systems, and is famous for being the light emitter of the photoprotein "aequorin".

Luciferases are proteins with enzymatic activity that, in the presence of ATP, oxygen and luciferin, catalyse the oxidation of the substrate i.e. luciferin and yield oxyluciferin in a reaction that results in the emission of a photon and emit light.



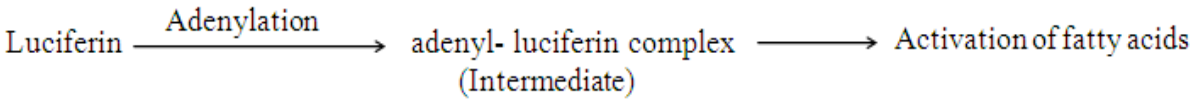
Use of luciferase reporters in plants have made use of genes from three sources:

1. Insect(*Photinus pyralis* of firefly)
2. Coelenterate *(Renilla reniformis* or sea pansy)
3. Bacteria (*Vibrio harveyi* and *Vibrio fishery*)

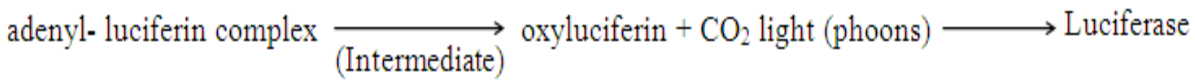
The genes used from these sources are- Firefly- FLUC gene, Sea pancy –RLUC gene, Bacterial- *lux* AB, Click beetle- cbLUC.The firefly luciferase from *Photinus pyralis* and the bacteria *lux* genes from *Vibrio harveyi* were the first to be adapted for use in plants.

1. Firefly luciferase-(FLUC)

Firefly (*Photinus pyralis*) luciferase is the best characterized bioluminescent reporter and continues to be the benchmark for imaging bioluminescence in transgenic plants.[44] Firefly luciferase is a euglobulin protein that catalyses the oxygenation of luciferin using ATP and molecular oxygen to yield oxyluciferin, a highly unstable, singlet-excited compound that emits light upon relaxation to its ground state.The luciferase enzyme is amonomeric protein (62 Kda) which can generate yellow green light through mono-oxygenation of beetle luciferin substrate.[45] The luciferin substrate is a benzothiazole found exclusively in fireflies (*P. pyralis* and *Luciola*) and in the presence of oxygen undergoes a Mg^{2+} and ATP-dependent reaction to produce dehydroluciferin, CO_2 and a photon of light.[46] The wavelength of photons emitted by FLUC centers around 560 nm. In fireflies, the oxygen required is supplied through a tube in the abdomen called the abdominal trachea. The reaction proceeds in two parts : 1) First step involves adenylation of luciferin, followed by the oxygenation of adenyl-luciferin. The adenylation step activates luciferin as an adenyl- luciferin complex, which is analogous to the activation of fatty acids by acyl-CoA ligases.



2) In the second step, luciferase acts as an oxygenase on adenyl- luciferin complex to produce oxyluciferin and carbon dioxide, the decay of oxyluciferin producing a photon of light.



The FLUC reporter has superior qualities as a non-invasive and non-destructive reporter for monitoring gene expression in vivo when it was used to characterize the expression pattern of the "gold standard" cauliflower mosaic virus 35S promoter in tissues of Nicotiana tabacum.[47,48] FLUC has since gained widespread use as a tool for monitoring gene expression changes in whole plants, protoplasts and detached tissues.[49,50,51] Other insect luciferases are currently being adapted for monitoring plant gene regulation[52] and will provide a new set of luciferase reporters with unique emission spectra and substrate specificities. New tools for plant molecular research are develop by using the new luciferase reporters genes.

2. Sea pancy luciferase- (RLUC)

Sea pansy luciferase from the anthozan coelenterate, *Renilla reniformis* – a bioluminescent soft coral, or sea pansy – has served as a valuable alternative to firefly luciferase. The RLUC enzyme exists in its active form as a nearly spherical single polypeptide monomer of 35 kDa.[53] Renilla luciferase requires only coelenterazine and oxygen. Renilla luciferase produces a blue light of 480nm.Renilla luciferase catalyzes the oxidative decarboxylation of coelenterazine substrates producing blue-green bioluminescence, oxyluciferin and CO_2.[53] The coelenterazine substrate, although chemically distinct from the FLUC substrate, is also referred to as luciferin. However, coelenterazine is only found in a range of salt water organisms such as sea urchins, shrimp and certain fish taxa [reviewed by.[54] The emission spectra of RLUC peaks around 480 nm,[53] which is a significant shift into the blue light region when compared to that of FLUC.

The luciferase (Renilla-luciferin) is closely associated with a luciferin-binding protein as well as a green fluorescent protein (GFP). Release of the luciferin (coelenterazine) from the luciferin binding protein triggered by Calcium . The luciferaes undergoes oxidation when substrate is available, where it is degraded to coelenteramide with a resultant release of energy. In the absence of GFP(green fluorescent protein), this energy would be released as a photon of blue light (peak emission wavelength 482 nm).

$$\text{Coelenterazine} + O_2 \longrightarrow \text{Coelenteramide} + CO_2 + \text{photon of light(482nm)}$$

The RLUC reporter was successfully used to report promoter activity in alfalfa protoplasts, tobacco, tomato and potato transgenic tissues and surpassed both the bacterial and firefly luciferase reporters in terms of overall sensitivity.[55] the RLUC reporter is combined with firefly luciferase for dual reporter applications, highly accurate quantification of gene expression can be obtained.[56,57] It displays unique properties that make it superior for some experimental systems, including a high substrate specificity and relatively simple assay conditions, requiring only dissolved oxygen and coelenterazine as substrates.

3. Bacterial luciferase-(*lux* AB)

Luminescent marine bacteria (*Vibrio harveyi* and *Vibrio fisheri*) have provided an additional source of bioluminescent genes that were first characterized around the same time as firefly luciferase.[58,59,60] The bacterial luciferase enzyme is a dimeric protein encoded by the *lux*A and *lux*B genes.[60,61] Bacterial luciferase oxidizes its substrate luciferin, which is a reduced riboflavin phosphate ($FMNH_2$), in association with a long chain aldehyde and oxygen molecule.[62]

$$FMNH_2 + O_2 + RCHO \rightarrow FMN + RCOOH + H_2O + light$$

Bacterial luciferin has been identified in free-living bacteria and in association with pyrosomes, as well as some squid and fish [reviewed by.[63] The emission spectra from *lux*AB luminescence peaks in the blue-green region (490 nm). Bacterial luciferase was initially ectopically expressedin *E. coli*[64] and later shown to serve as a useful reporter of promoter function in several plant systems, including tobacco, carrot and populus.[65,66,67,68] In bacteria and plant the *lux*AB reporter system has proven effective for in vitro assays, it has not gained widespread use for in vivo imaging in plants.

4.Click beetle luciferase- (CbLUC)

The luminous click beetle luciferase which is related to the firefly luciferase and has emerged as a new alternative for co-reporter assays. CbLUC bioluminescence is unusual in that individual beetle specimens contain two sets of light-emitting organs that can differ in the color of light emitted.The altered amino acids were found to be responsible for the shift in emission spectra from green to orange (544 to 593 nm). Green (CBG68luc and CBG99luc) or red (CBRluc) regions of the visible spectrum (537 to 613 nm).[69] The common substrate

share by green and red click beetle luciferases, but have emission spectral maxima separated by ~75 nm. This allows light from the two reporters to be independently quantified using filters that discriminate between the two emission maxima.

All luciferases are classified as oxidoreductases (EC 1.13.12.-) (oxidoreductase is an enzyme that catalyzes the transfer of electron from one molecule to another molecule) meaning they act on single donors with incorporation of molecular oxygen. Luciferases are from many diverse protein families which are unrelated, there mechanism are also unknown, as any mechanism depends on both luciferase and luciferin combination. However, luciferase-luciferin reactions systemrequired moleculat oxygen at some stage of the reaction. Nearly all energy released during the reaction is transformed into light.

lux GENES

The set of *lux* genes in bacteria coded forluciferin-luciferase system. five such genes (*lux*CDABEG) have been identified as active in the emission of visible light from the *V. fischeri*, and two genes (*lux*R and *luxI*) are involved in regulating the operon(Fig-01). The bacterium *Vibrio fischeri*, operon is most well studied operon whose operon-produced luciferase produces a yellowish colored bioluminescence of about 490 nm.

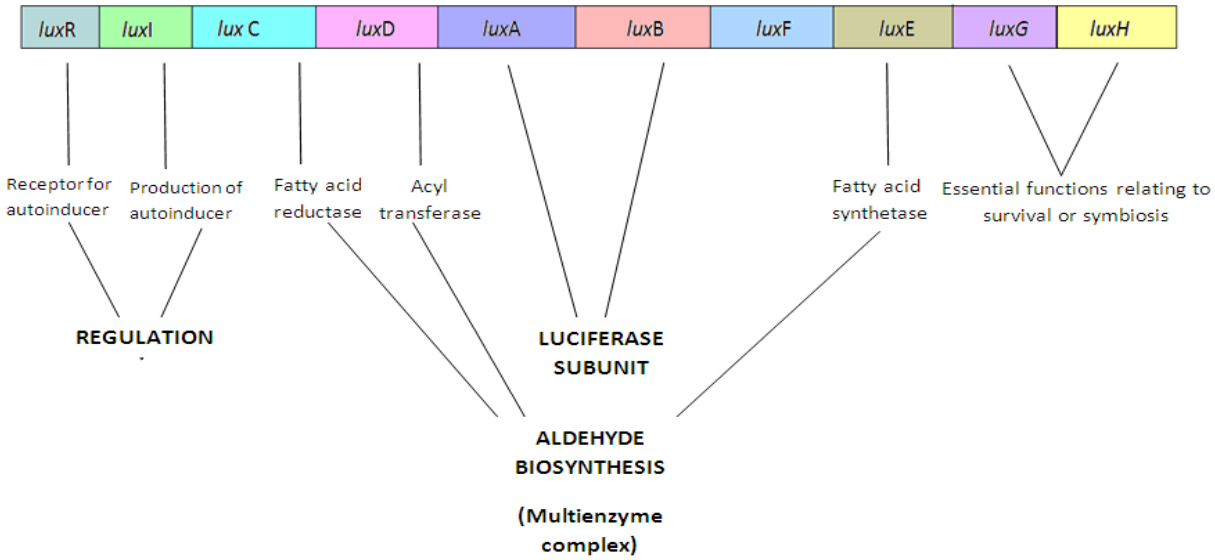


Fig-01: Organization of *lux* genes.

lux GENE ORGANIZATION

*lux*Structural Genes (*lux*CDABFE)

lux genes have been cloned from a numerous luminescent bacterial strains including *V. fischeri, V. harveyi, P. phosphoreum, P. leiognathi,* and *X. luminescens.* In all cases, *lux*A and

*lux*B genes coding for the luciferase subunits and *lux*CDE genes coding for the fatty acid reductase complexhave been identified. In addition, other *lux* genes (*lux*F, *lux*G, *lux*H, *lux*I, and *lux*R) as well as unlinked *lux* gene loci have been identified in specific luminescent strains. Outlined in Fig. 2 are the *lux* gene organizations for different luminescent bacterial strains.

The order of the genes coding for the luciferase (*lux*AB) and fatty acid reductase (*lux*CDE) enzymes is the same [*lux*CDAB(F)E] in all operons, with *lux*C and *lux*D, which code for the reductase and transferase polypeptides of the fatty acid reductase, flanking the luciferase genes upstream and *lux*E, which codes for the synthetase, being downstream. An additional gene (*lux*F) is located between *lux*B and *lux*E in most but not all Photobacterium species and is missing in the *Vibrio* and *Xenorhabdus lux* systems. As the sequences of this gene, denoted as *luxG*[70] and *luxN*[71] in *P. leiognath*i 554 and *P. leiognathi* 721, respectively, are virtually identical and homologous to the*lux*F gene of *P.phosphoreum*,[72] the latter designation has been adopted for this gene in all species. The recent determination of the nucleotide sequence of the *lux* genes of a different *P. leiognathi* strain (ATCC 25521),[73] isolated directly from the light organ of a pony fish, has shown that the *lux*F gene is absent in some *Photobacterium* species (Fig. 02). As the exact origin of the other *P. leiognathi* strains is unknown, it is possible that the presence of the*lux*F gene coincides with a specific environmental niche of *P. phosphoreum* and some strains (P1,) of *P. leiognathi.*

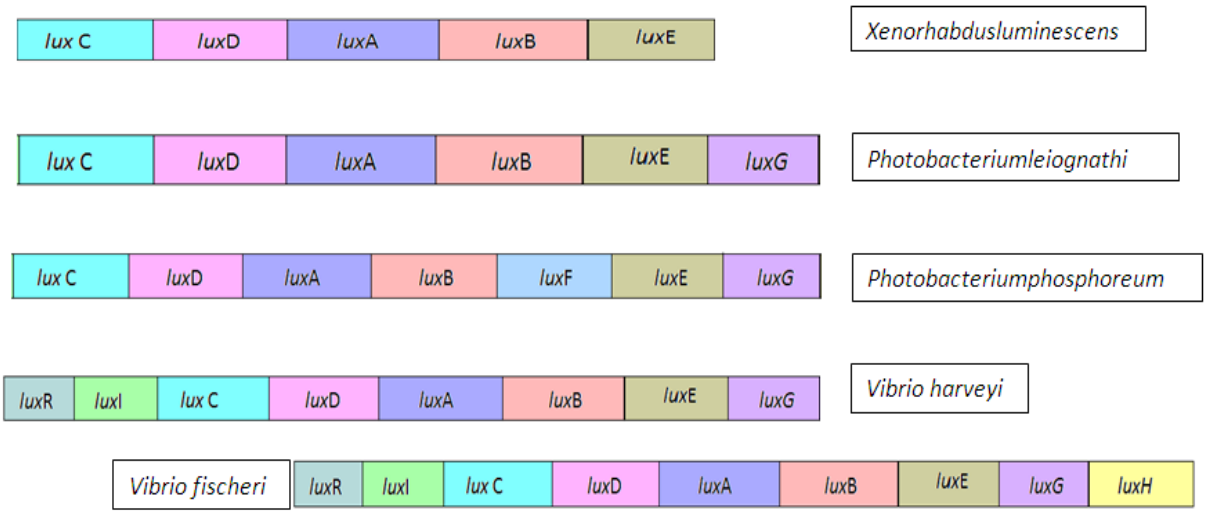


Fig. 02: *lux* gene organization for *X. luminescens (Xl), P. leiognathi (P1), P. phosphoreum (Pp), V. harveyi (Vh), and V. fischeri (Vf).*

Upstream and Regulatory Genes

Upstream of the *lux*C gene in the *V. fischeri lux* system are two regulatory genes, *lux*I and *lux*R, which are missing or not located at these positions in other luminescent species. The *lux*I gene is located immediately in front of *lux*C and is part of the same operon (right operon). The *lux*I gene product is believed to be involved in production of the autoinducer required for induction of the luminescence system.[74,75] Mutations in this gene can be complemented by the addition of the autoinducer for the *V. fischeri lux* system. Transcribed in the opposite direction on the left operon is the *lux*R gene. Mutations in this region block expression of the *V. fischeri lux* system.[74] The *lux*R gene product has been proposed to function as a receptor for the autoinducer.[76] This complex then stimulates transcription of the right operon.

Downstream *lux* Genes

Downstream of *lux*E is the *lux*G gene in the *lux* operons of all marine bacteria so far investigated. This gene in the *Vibrio* systems has been shown to be encoded on mRNA that is induced during the development of luminescence.[77,78] The function of the *lux*G gene product is unknown. Transposon insertions in this region of *V. fischer*i or *V. harveyi* have not been found that disrupt the expression or regulation of the luminescence system.[74,79] However, the presence of this gene could be related to the physiological habitat of the marine luminescent bacteria as *lux*G is not present at this location in *Xenorhabdus* species. An additional lux gene (*lux*H) is found downstream of *lux*G in the *V. harveyilux* system but not in the *V. fischeri*or *P.leiognathi lux* systems. As transposon mutagenesis has failed to produce any *Lux*-phenotypes with insertions in this gene,[79] it seems likely that the gene product is not involved in the general regulation or expression of the *lux* system in *V. harveyi*. It is possible that the gene product modulates the expression of the system under specific physiological conditions that may be related to the natural habitat of the bacteria. Alternately, these downstream genes may be required for an essential function relating to the survival and/or symbiosis of the bacteria in the marine environment.

FUNCTIONS ENCODED BY THE *lux* GENES

Luciferase

The light-emitting reaction in bacteria involves the oxidation of reduced long chain fatty aldehyde and riboflavin phosphate ($FMNH_2$) with the emission of blue-green light. For the reaction catalyzed by bacterial luciferases the term luciferin has generally not been used to

refer as the substrates but instead of luciferin the long chain fatty aldehyde and riboflavin phosphate ($FMNH_2$) are used as a substrate. Because the structure of the substrates are relatively simple ($FMNH_2$ and RCHO) and are part of and closely related to the normal metabolites in the cell.

This reaction is as follows:

$$FMNH_2 + RCHO + 0_2 \longrightarrow FMN + H_20 + RCOOH + hv\ (490\ nm)$$

The reaction is highly specific for $FMNH_2$. Modification of the flavin ring or removal of the phosphate group decreases the activity significantly.[80] The natural aldehyde for the bioluminescence reaction is believed to be tetradecanal on the basis of identification of this compound in lipid extracts, the preference for tetradecanal by luciferases at low (nonsaturating) substrate concentrations, and the specificity of the *lux*-specific fatty acid reductase system, which catalyzes the synthesis of the fatty aldehyde substrate.[81,82,83,4,85]

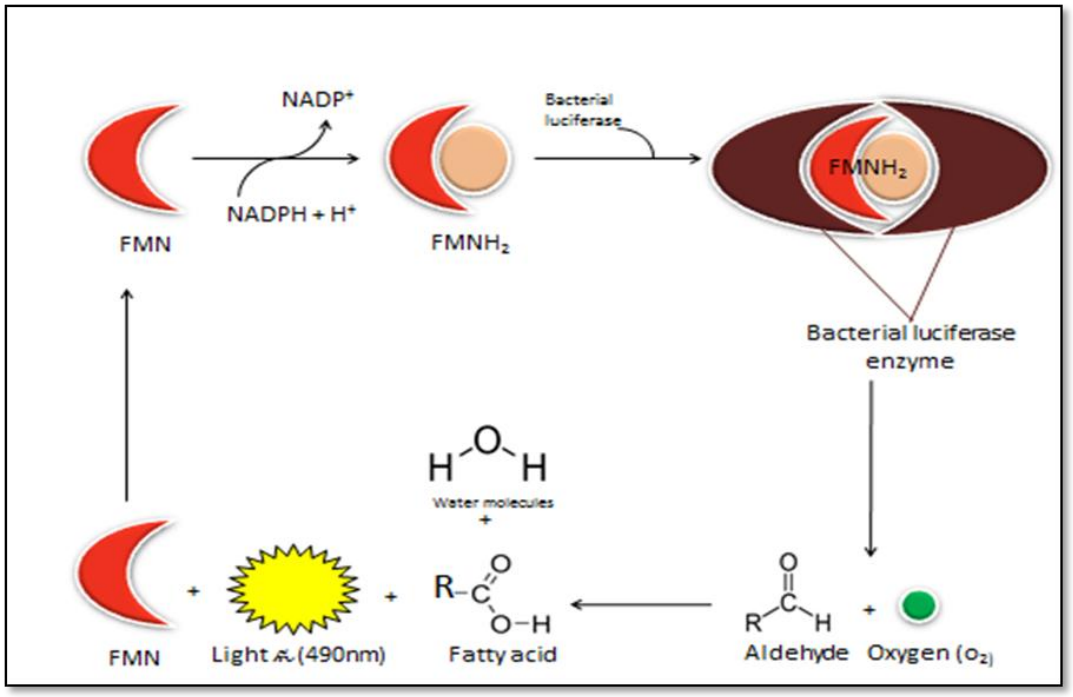


Fig-03: Reaction catalyzed by bacterial luciferase.

However, differences in aldehyde specificity do exist among different bacterial luciferases. Particularly noteworthy are the high luminescent responses of *V. harveyi* and *X. luminescens* luciferases to nonanal and decanal at saturating substrateconcentrations, whereas higher light intensity can be obtainedwith dodecanal for *P. phosphoreum*, *P. leiognathi*,and *V. fischeri* luciferases.[86,87,88,89] This propertymay be very important in terms of expression of light

emission in vivo in prokaryotic and eukaryotic cells missing the aldehyde substrate, because decanal appears to cross the cell membrane much more readily than longer-chain aldehydes do. The mechanism of the bioluminescence reaction catalyzed by luciferase and the intermediates in the reaction have been studied extensively[90,91] primarily the reduced flavin, $FMNH_2$,bound to the enzyme form enzyme substrate complex this enzyme substrate complex reacts with the 0_2 to form a 4a-peroxyflavin. This complex interacts with aldehyde to form a highly stable intermediate, which decays slowly, resulting in the emission of light along with the oxidation of the substrates.(Fig-03) Luciferase undergoes only a single catalytic cycle in most assays, because the rate of chemical oxidation of $FMNH_2$ is higher than the turnover rate of luciferase in the bioluminescence reaction. Consequently, the decay of luminescence with time is a first-order process and reflects the turnover number of the enzyme under the assay conditions. Halftimes for turnover are dependent on experimental conditions, including the particular luciferase and aldehyde. Forexample, *X. luminescens* luciferase has the lowest turnover rates, with half times of 3 and 20 s with decanal and dodecanal,[89] respectively. Bacterial luciferase is a heterodimeric enzyme of 77 kDa, composed of α and β-subunits with molecular masses of 40and 37 kDa, respectively.(fig-04) The two polypeptides, encoded on closely linked adjacent genes, *lux*A and *lux*B in the *lux* operon, appear to have arisen by gene duplication.[92] since there is about 30% identity in the amino acid sequence between the a and 3 subunits of all bacterial luciferases.[93,94,89] Luciferase is produced in very large amounts at least in the marine bacteria, and the *lux*A and l*ux*B genes can readily be expressed in *Escherichia coli*, providing abundant sources of protein for purification and/or application.

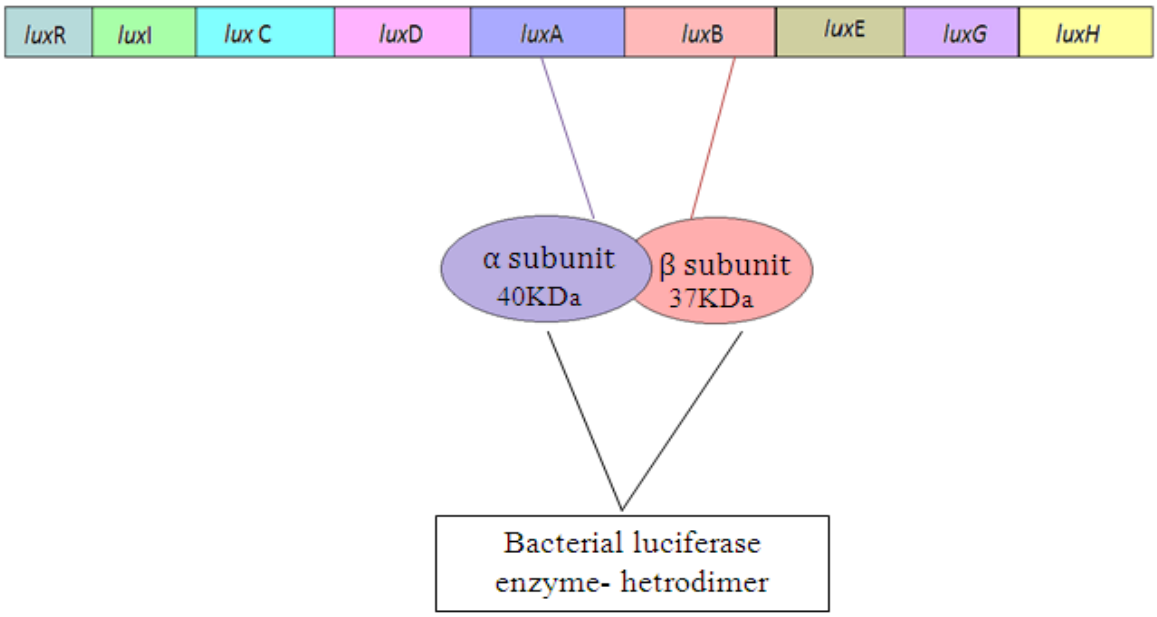


Fig-04: Bacterial luciferase enzyme coded by lux A &lux B genes.

Aldehyde Biosynthesis

The synthesis of aldehydes for the bioluminescence reaction is catalyzed by a multienzyme fatty acid reductase complex containing three proteins, a reductase, a transferase, and a synthetase[95,96,97] These three polypeptides are encoded by *lux*C, *lux*D, and *lux*E, respectively, found in the *lux* operons of all luminescent bacteria.[98,99,100,101] The transferase subunit catalyzes the transfer of activated fatty acyl groups to water as well as other oxygen and thiol acceptors, with the enzyme being acylated during the course of the reaction.

$$RCOX + HOH\ (HSR') \longrightarrow RCOOH\ (RCOSR') + XH$$

Maximum rates of cleavage occur with the substrates tetradecanoyl- acyl carrier protein (ACP), tetradecanoyl coenzyme A, and p-nitrophenyltetradecanoate, demonstrating the high specificity for 14-carbon acyl groups.[101] The ability to cleave acyl-ACP in extracts of luminescent bacteria and in *E. coli* transformed with the *lux*D gene appears to be exclusively catalyzed by the transferase subunit, providing a highly specific assay for detection of the expression of *lux*D.[102] The primary reaction catalyzed by the fatty acid reductase complex is the reduction of fatty acids to aldehydes:

$$RCOOH + ATP + NADPH \longrightarrow NADP + AMP + PP; + RCHO$$

This reaction is catalyzed by the reductase and synthetase components. The synthetase activates the fatty acid, resulting in the formation of a fatty acyl-AMP intermediate that is tightly bound to the enzyme. In the presence of the reductase, the acyl group is transferred first to the synthetase and then to the reductase before being reduced by NADPH to aldehyde.[103] The reductase component can also directly reduce acyl coenzyme A and has often been referred to as acyl coenzyme A reductase.[95,96]

These polypeptides have molecular masses of 54 kDa(reductase), 42 kDa (synthetase), and 33 kDa (transferase) in the different luminescent strains and form a multi enzymecomplex of 5 x 105 Da in *P. phosphoreum.*[104] This complex consists of a central tetramer of reductase subunits, each of which interacts with a synthetase subunit that in turn binds weakly to the transferase subunits.

Flavoproteins

A flavoprotein with subunits of molecular mass of about 24 kDa[105] appears to be part of the *lux* systems of most *Photobacterium* species. Recently, the flavin prosthetic group was suggested to be flavin mononucleotide covalently linked at the 6-position to the β or α carbon of tetradecanoicacid.[106] The function of this protein, encoded by *lux*F, is unknown; however, it is homologous in amino acid sequence to the luciferase subunits and thus appears to have arisen by gene duplication.[107] Its function may be related to the physiological and/or environmental niche of certain *Photobacterium* species, because it has not been found in *Vibrio or Xenorhabdus* strains and is not necessary for expression of light by the *Photobacterium lux* system in *E. coli*.

Regulatory and Other Functions

New *lux* genes, *lux*G and *lux*H, coding for 25-kDa polypeptidesof unknown function have just been identified.[108,109] *lux*G has been found in all strains of luminescent bacteria except *Xenorhabdus* strains and consequently may code for a function associated with the marine environment. *lux*H is part of the *V. harveyi* but not the *V. fischerilux* operon and therefore may code for a polypeptide involved in a specific role related to the luminescence system of *V.harveyi*. New *V. harveyi lux* gene loci have recently been identified that code for regulatory functions necessary for luminescence.[79,110] Regulatory functions, encoded by *lux*R and *lux*I of *V. fischeri* and involving the synthesis of a receptor protein and autoinducer, respectively, have also been characterized[100,111] and are described in a later section.

Functions Related to Luminescence

Lumazine and yellow fluorescence protein

Lumazine and yellow fluorescence protein have been isolated from *Photobacterium* and *Vibrio* species, respectively. lumazine and yellow fluorescence proteins affect the wavelength of the emitted light. The lumazine proteins shift the color of the light to shorter wavelengths than 490 nm, and as a result it has been proposed that the initial excited state in the luminescence reactions cannot be a flavin derivative, which would be expected to emit light at higher wavelengths [91] A strain of *V. fischeri* that emits yellow light at 540 nm has also been investigated, and a yellow fluorescence protein has been identified that is responsible for the change in the color of the light [112,113] At present, the genes coding for these proteins have not been identified.

Aldehyde degradation

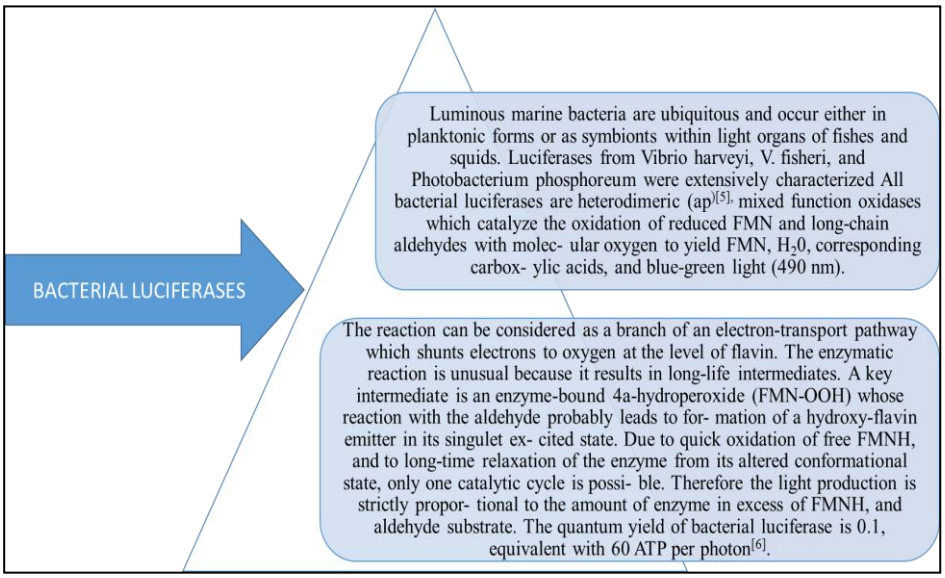
An aldehyde dehydrogenase with a high specificity for NADP has been purified from *V. harveyi*.[114] Although this enzyme can catalyze the synthesis as well as the oxidation of aldehydes and has specificity and properties similar to those of the reductase component involved in aldehyde synthesis, its expression is under different control from that of the fatty acid reductase polypeptides encoded by *lux*C, *lux*D, and *lux*E.[115] Further work is required to clearly establish its specific relationship to the luminescence system.

BACTERIAL AND FIREFLY LUCIFERASE GENES IN TRANSGENIC PLANTS

BACTERIAL LUCIFERASES

Luminous marine bacteria are ubiquitous and occur either in planktonic forms or as symbionts within light organs of fishes and squids. Luciferases were extensively characterized from *Vibrioharveyi*, *V. fisheri*, and *Photobacterium phosphoreum.*[116,117] All bacterial luciferases are heterodimeric (ap), mixed function oxidases which catalyze the oxidation of reduced FMN and long-chain aldehydes with molecular oxygen to yield FMN, H_20, corresponding carboxylicacids, and blue-green light (490 nm). The reaction can be considered as a branch of an electron-transport pathway which shunts electrons to oxygen at the level of flavin. The enzymatic reaction is unusual because it results in long-life intermediates. A key intermediate is an enzyme-bound 4a-hydroperoxide (FMN-OOH) whose reaction with the aldehyde probably leads to formation of a hydroxy-flavin emitter in its singulet excited state. Due to quick oxidation of free FMNH, and to long-time relaxation of the enzyme from its altered conformational state, only one catalytic cycle is possible. Therefore the light production is strictly proportional to the amount of enzyme in excess of FMNH, and aldehyde substrate. The quantum yield of bacterial luciferase is 0.1, equivalent with 60 ATP per photon (flow chart-01). The aldehyde-binding site of the luciferase α-subunit contains an essential sulfhydryl group close to the αβ subunit interphase. The non-catalytic P subunit is required for proper folding and conformational change of the α-subunit during interaction with the flavin molecule. Structural mutations in both enzyme subunits, as well as various flavin analogs, can alter the emission spectra between 490 and 535 nm.[118,119,120,121,122] lumazine protein forms a complex with luciferase and, by energy transfer from the flavin to the secondary emitter 6,7-dimethyl-8-(l1-D-ribityll-lumazine, blue light (475 nm) is emitted.[123] In *Vibrio fischeri* strain Y-1 energy transfer occurs from excited flavin intermediates to a flavin containing secondary emitter protein, termed yellow fluorescent protein (YFP), causes a yellow shift (534 nm) in the emission of light. In the

presence of NAD(P)H-FMN oxydoreductase, an enzyme supplying reduced FMN in luminous bacteria, the addition of YFP does not only shift the colour but also increases the intensity of total light emitted three- to four-fold.



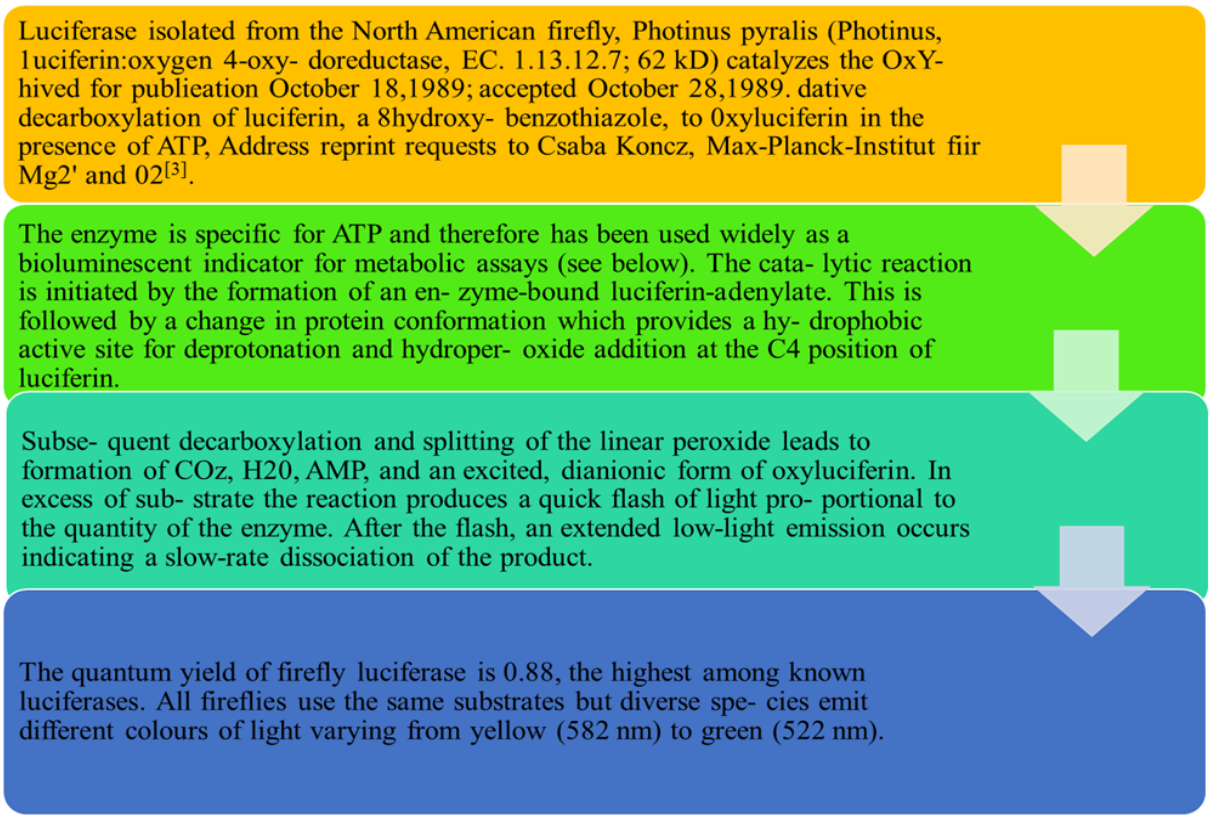
Flow chart- 01

Bacterial luciferase

FIREFLY LUCIFERASE: Refer (Flow chart-02).

Flow chart-02

Firefly luciferase



Low pH and divalent cat- ions shift the light emission to red, indicating that the conformational change of the enzyme and alterations in its structure play an important role in determining the energy of the excited product and thus the colour of emitted light

Recently, lu- ciferase cDNAs were cloned from the Japanese firely [Masuda et al., 19891 and from beetles [Wood et al., 19891 which produce different colours of light when expressed in E. coli. The identification of amino acid exchanges between these enzymes should pinpoint pep- tide domains involved in enzyme-substrate interaction, as well as open the way to engineer novel luciferases

luciferase can also catalyze the addition of adenylated dihydroluciferin to CoA, in a reaction similar to that catalyzed by fatty acyl CoA synthases. Since this reaction is CoA-specific, it is probably not a coincidence that the enzyme shows a significant homology to other CoA-specific enzymes, such as plant 4-coumarate-CoA ligase[4].

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EXPRESSION OF LUCIFERASE GENES IN TRANSGENIC PLANTS

Refer flow chart- 03 and Fig no-04

Transgenic plants are plants that have been genetically engineered, a breeding approach that uses recombinant DNA techniques to create plants with new characteristics. They are called as (GMO) genetically modified organism

Application of luciferase genes in eucaryotes is still in its infancy, but will certainly lead to a burst of novel experimental systems soon. Among eukaryotic organ- isms the expression of luciferase genes was first dem- onstrated in plants. Linked to the romoter of the Cau- liflower Mosaic Virus 35s transcript, the firefly luciferase gene was introduced into carrot protoplasts by electroporation and into tobacco by Agrobacterium-mediated transformation.

In addition to in vitro mea- surements of luciferase activity in transient assays, the luciferase-mediated light emission was visualized by autoradiography of cell suspensions and by contact ex- posure of a transgenic plant to a photographic emulsion[[8].

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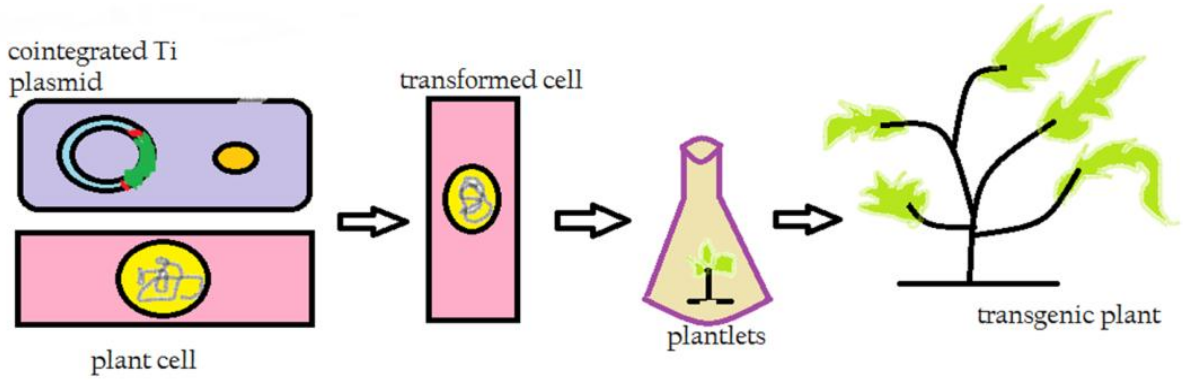

Flow chart-03 and Fig-04 Expression of luciferase genes in transgenic plants

LUCIFERASE ASSAYS

Light can be monitored visually, photographically, or electronically at different sensitivities. A great variety of methods for detection and measurement of bioluminescence have been described.[124] Following pioneering work by several laboratories on the purification and immobilization of luminescent enzymes, luciferases found a wide range of applications in most areas of life sciences. A particular advantage of luciferase assays is their ease, sensitivity, and efficiency. Practically any reaction which can be linked to measurement of ATP, NAD(P), FMN, fatty acids, or aldehydes can be monitored by firefly and bacterial luciferases. Special features of other luminescent proteins, such as aequorin also allow one to measure Ca^{2+}-mediated reactions. The range of in vitro assays extends from clinical, microbial detection of pathogens to biochemical assays of enzymes, cofactors, and substrates, to mutagenicity tests, to detection of steroid hormones and insect pheromones, and to the measurement of membrane transport and organellar functions.[125,126,127,128] Recent advances in selective modification of a reactive sulfhydryl group of bacterial luciferase and in the synthesis of firefly luciferins derivatized at the 6-position led to the general application of luciferases in immunoassays, protein immuno-blotting, and non-radioactive nucleic acid hybridization.[129,130]

CONCLUSION

The production of electricity for human use offers numerous conveniences but it can also harm the environment. So there is a need to develop the tool for bio light. There are obvious environmental benefits to using the bio lights. The aim is to transform an electric source of light into a biological source of light using bioluminescence. It was obvious that this superpower that some living organisms have could be the solution to the tremendous

economic and ecological challenges of urban lighting.It is not to entirely replace electric light with bioluminescence but to use it as an alternative solution. It is an alternative solution to:

1. Reduce the 19% share of electricity used in the world to light, which accounts for 5% of global greenhouse gas emission.
2. To provide light to people or places which do not have access to electricity i.e. used in remote location where it is too complicated or expensive.

Bioluminescent lighting may soon be used as an alternative to electrical lighting to illuminate car parks and light up buildings or streets at night the light from the sea! A living lighting energy, coming directly from nature, at the cross roads of bio mimicry and synthetic biology, ready to revolutionize the way to produce, consume and light up!

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REFRENCES

1. Dubois, R. 1887. Note sur la fonctionphotogenique chez les Pholades. C.R. Soc. Biol. Ser, 8(3): 564-568.
2. Boyle, R. 1668. Experiments concerning the relation between light and air in shining wood and fish. Philos. Trans. R. Soc. London, 2: 581-600.
3. Dubois, R. 1887. Note sur la fonctionphotogenique chez les Pholades. C.R. Soc. Biol. Ser., 8(3): 564-568.
4. Campbell, A. K. 1989. Living light: biochemistry, function and biomedical applications. Essays Biochem, 24: 41-76.
5. Harveyi, E. N. 1952. Bioluminescence. Academic Press, Inc., New York.
6. Hastings, J. W. 1986. Bioluminescence in bacteria and dinoflagellates, p. 363-398.In A. J. Govindjee and D; C. Fork (ed.), Light emission by plants and bacteria.Academic Press, Inc., New York.
7. Hastings, J. W., J. Makemson, and P. V. Dunlap. 1987. How are growth and luminescence regulated independently in light organ symbionts? Symbiosis, 4: 3-24.

8. Hastings, J. W., C. J. Potrikas, S. C. Gupta, M. Kurfurst, and J. C. Makemson. 1985. Biochemistry and physiology of bioluminescent bacteria. Adv. Microbiol. Physiol, 26: 235-291.
9. Hastings, J. W. 1986. Bioluminescence in bacteria and dinoflagellates, p. 363-398.In A. J. Govindjee and D; C. Fork (ed.), Light emission by plants and bacteria.Academic Press, Inc., New York.
10. Baumann, P., L. Baumann, M. Woolkalis, and S. Bang. 1983. Evolutionary relationships in *Vibrio* and *Photobacterium*. A basis for a natural classification.Annu. Rev. Microbiol, 37: 369-398.
11. Nealson, K. H., and J. W. Hastings. 1979. Bacterial bioluminescence: its control and ecological significance. Microbiol. Rev, 43: 496-518.
12. Ferri, S., R. Soly, and C. Lee. Unpublished data.
13. Hada, H. S., J. Stemmler, M. L. Grossbard, P. A. West, C. J. Potrikus, J. W. Hastings, and R. R. Colwell. 1985. Characterization of non-O1-serovar *Vibrio*cholerae (*Vibrio albensis*). Syst. Appl. Microbiol, 6: 203-209.
14. Jensen, M. J., B. M. Tebo, P. Baumann, M. Mandel, and K. H. Nealson. 1980. Characterization of Alteromonashanedai (sp. nov.), a nonfermentative luminous species of marine origin. Curr.Microbiol.
15. Baldwin, T. O., T. Berends, T. A. Bunch, T. F. Holzman, S. K. Rausch, L. Shamansky, M. L. Treat, and M. M. Ziegler. 1984. Cloning of the luciferase structural genes from *Vibrio harveyi* and expression of bioluminescence in *Escherichia coli*. Biochemistry, 23: 3663-3667.
16. Belas, R., A. Mileham, D. Cohn, M. Hilmen, M. Simon, and M. Silverman. 1982. Bacterial bioluminescence: isolation and expression of the luciferase genes from *Vibrio harveyi*. Science.
17. Delong, E. F., D. Steinhauer, A. Israel, and K. H. Nealson. 1987. Isolation of the *lux* genes from *Photobacteriumleiognathi* and expression in *Escherichia coli*. Gene, 54: 203-210.
18. Engebrecht, J., K. H. Nealson, and M. Silverman. 1983. Bacterial bioluminescence: isolation and genetic analysis of functions from *Vibrio fischeri*. Cell, 32: 773-781.
19. Evans, J. F., S. McCracken, C. M. Miyamoto, E. A. Meighen, and A. F. Graham. 1983. In vitro synthesis of the subunits of bacterial luciferase in an *Escherichia coli* system. J. Bacteriol, 153: 543-545.

20. Frackman, S., M. Anhalt, and K. H. Nealson. 1990. Cloning, organization, and expression of the bioluminescence genes of *Xenorhabdusluminescens*. J. Bacteriol, 172: 5767-5773.
21. Mancini, J. A., M. Boylan, R. R. Soly, A. F. Graham, and E. A. Meighen. 1988. Cloning and expression of the *Photobacteriumphosphoreu*m luminescence system demonstrates a unique *lux*gene organization. J. Biol. Chem., 263: 14308-14314.
22. Szittner, R., and E. Meighen. 1990. Nucleotide sequence, expression and properties of luciferase coded by *lux* genes from a terrestrial bacterium. J. Biol. Chem, 265: 16581-16587.
23. Cohn, D. H., R. C. Ogden, J. N. Abelson, T. 0. Baldwin, K. H Nealson, M. I. Simon, and A. J. Mileham. 1983. Cloning of the *Vibrio harveyi* luciferase genes: use of a synthetic oligonucleotide probe. Proc. Natl. Acad. Sci. USA, 80: 120-123.
24. De Wet, J. R., K. V. Wood, D. R. Helinski, and M. DeLuca. 1985. Cloning of firefly luciferase cDNA and the expression of VOL. 55, 1991 active luciferase in *Escherichia coli*. Proc. Natl. Acad. Sci. USA, 82: 7870-7873.
25. Wood, K. V., Y. A. Lam, H. H. Seliger, and W. D. McElroy. 1989. Complementary DNA coding click beetle luciferases can elicit bioluminescence of different colors. Science, 244: 700-702.
26. Kurose, K., S. Inouye, Y. Sakaki, and F. I. Tsuji. 1989. Bioluminescence of the Ca"+ binding photoproteinaequorin after cysteine modification. Proc. Natl. Acad. Sci. USA, 86: 80-84.
27. Prasher, D. C., C. 0. McCann, M. Longlaru, and M. J. Cormier. 1987. Sequence comparisons of complementary DNAs encoding aequorinisotypes. Biochemistry, 26: 1326-1332.
28. Thompson, E. M., S. Nagata, and F. I. Tsuji. 1989. Cloning and expression of cDNA for the luciferase from the marine ostracod Vargulahilgendorfii. Proc. Natl. Acad. Sci. USA, 86: 6567-6571.
29. Owens, Leigh; Busico-Salcedo, Nancy (2006). "*Vibrio harveyi*: Pretty Problems in Paradise (Chapter 19)". In Thompson, Fabiano; Austin, Brian; Swings, Jean. The Biology of Vibrios. ASM Press.
30. Jump up^ Austin B & Zhang XH (2006). "*Vibrio harveyi*: a significant pathogen of marine vertebrates and invertebrates". Letters in Applied Microbiology, 43(2): 119–214. doi:10.1111/j.1472-765X.2006.01989.x. PMID 16869892.

31. Madigan M, Martinko J (editors) (2005). Brock Biology of Microorganisms (11th ed.). Prentice Hall. ISBN 0-13-144329-1.
32. Bergey, DH (1994). Holt, JG, ed. Bergey's Manual of Determinative Bacteriology (9th ed.). Baltimore: Williams & Wilkins.
33. Hoi Ho, Thong Huy Do, Tony Tran Ho, Derek Lee. "*Vibrio* infections." 2007 January.Emedicine Specialties. http://www.emedicine.com/med/topic2375.htm.
34. F.L Thomposon.T. lida. J. Swings. ‘’Biodiversity of Vibrios” .Microbial mol bio Rev. September 2004; 68.
35. Nonbioluminescent Strains of *Photobacteriumphosphoreum*Produce the Cell-to-Cell Communication Signal N-(3-Hydroxyoctanoyl)homoserine Lactone. Ncbi .pubMed.
36. K.H. Nealson, J.W. Hastings, “Bacterial Bioluminescence: its control and ecological significance”.
37. J.Mancinic, M.Boylan . R. Soly. S.Ferri. R. Szitter.E. Meighen.” Organization of *lux* genes of *photobacteriumphosphoreum*”. Journal of Bioluminescence.
38. T.Sakaguchi, “Rapid and onsite BOD sensing system using luminous bacterial cell immobilizied chip”. Biosensors and bioelectronics, 2007; 22(7): 1345-1350.
39. Blackburn, MB; Domek, JM; Gelman, DB; Hu, JS (2005). "The broadly insecticidal *Photorhabdusluminescens* toxin complex a (Tca): Activity against the Colorado potato beetle, Leptinotarsadecemlineata, and sweet potato whitefly, Bemisiatabaci". Journal of Insect Science, 5: 32. PMC 1615239. PMID 17119614.Archived from the original on 2013-12-02.Retrieved 2016-11-11.40.
40. Hu, K; Webster, JM (2000). "Antibiotic production in relation to bacterial growth and nematode development in Photorhabdus--Heterorhabditis infected Galleria mellonella larvae". FEMS Microbiology Letters, 189(2): 219–23. doi:10.1111/j.1574-6968.2000.tb09234.x. PMID 10930742.41.
41. Durham, Sharon (2001-05-29). "Students May Have Answer for Faster-Healing Civil War Wounds that Glowed". Agricultural Research Service.
42. Kwok, Roberta (21 January 2012). "New Scientist - 21 January 2012 -Driller Killer". New Scientist.
43. Soniak, Matt (2012-04-05). "Why Some Civil War Soldiers Glowed in the Dark". Mental Floss.44.Travis J, McElroy W (1966) Isolation and sequence of an essential sulfhydrylpeptide at the active site of firefly luciferase. Biochemistry 5, 2170-217645.Wood K, de Wet J, Dewji N, DeLuca M (1984) Biochemical and BiophysicalResearch Communications 124, 592-59646.

44. Hastings J (1998) Bioluminescence. In: Sperelakis N (Ed) Cell Physiology,Academic Press, New York, pp 984-100047.
45. Howell S, Ow D, Schneider M (1989) Use of the firefly luciferase gene as reporter of gene expression in plants. In: Gelvin S, Schilperoort R, Verma D(Eds) Plant Molecular Biology Manual, Kluwer Academic Publishers, Belgium, pp 1-1148.
46. Ow D, Wood K, DeLuca M, de Wet J, Helsinki D, Howell S (1986) Transientand stable expression of the firefly luciferase gene in plant cells and transgenicplants. Science, 234: 856-859.
47. Millar AJ, Short SR, Chua NH, Kay SA (1992a) A novel circadian phenotype based on firefly luciferase expression in transgenic plants. Plant Cell 4: 1075-1087.
48. Van Leeuwen W, Hagendoorn M, Ruttink T, Poecke R, van der Plas L, vander Krol A (2000) The use of the luciferase reporter system for in planta gene expression studies. Plant Molecular Biology Reporter, 18: 143a-143t.
49. Southern MM, Brown PE, Hall A (2006) Luciferases as reporter genes.Methodsin Molecular Biology, 323: 293-305.
50. Viviani V (2002) The origin, diversity, and structure function relationships of insect luciferases. Cellular and Molecular Life Sciences, 59: 1833-1850.
51. Matthews J, Hori K, Cormier M (1977) Purification and properties of Renillareniformisluciferase. Biochemistry, 16: 85-91.
52. Greer LF, 3rd, Szalay AA (2002) Imaging of light emission from the expression of luciferases in living cells and organisms: a review. Luminescence, 17: 43-74.
53. Mayerhofer R, Langridge WHR, Cormier MJ, Szalay AA (1995) Expression of recombinant Renillaluciferase in transgenic plants results in high levels of light emission. Plant Journal, 7: 1031-1038.
54. Matsuo N, Minami M, Maeda T, Hiratsuka K (2001) Dual luciferase assay for monitoring transient gene expression in higher plants. Plant Biotechnology, 18: 71-75.
55. Cazzonelli CI, Velten J (2006) Anin vivo, luciferase-based, Agrobacteria-infiltration assay system: implications for post-transcriptional gene silencing. Planta, 224: 582-597.
56. Baldwin TO, Berends T, Bunch TA, Holzman TF, Rausch SK, ShamanskyL, Treat ML, Ziegler MM (1984) Cloning of the luciferase structural genes from *Vibrio harveyi*and expression of bioluminescence in *Escherichia coli*. Biochemistry, 23: 3663-3667.
57. Engebrecht J, Silverman M (1984) Identification of genes and gene products necessary for bacterial bioluminescence. Proceedings of the National Academyof Sciences USA, 81: 4154-4158.

58. Cohn DH, Mileham AJ, Simon MI, Nealson KH, Rausch SK, Bonam D, Baldwin TO (1985) Nucleotide sequence of the *lux*Agene of *Vibrio harveyi* and the complete amino acid sequence of the alpha subunit of bacterial luciferase. The Journal of Biological Chemistry, 260: 6139-6146.
59. Johnston TC, Thompson RB, Baldwin TO (1986) Nucleotide sequence of the *lux*B gene of *Vibrio harveyi* and the complete amino acid sequence of the beta subunit of bacterial luciferase. The Journal of Biological Chemistry, 261: 4805-4811.
60. Hastings JW, Baldwin TO, Nicoli MZ (1978) Bacterial luciferase: assay, purification and properties. Methods in Enzymology, 57: 135-152.
61. Greer LF, 3rd, Szalay AA (2002) Imaging of light emission from the expression of luciferases in living cells and organisms: a review. Luminescence, 17: 43-74.
62. Baldwin TO, Berends T, Bunch TA, Holzman TF, Rausch SK, ShamanskyL, Treat ML, Ziegler MM (1984) Cloning of the luciferase structural genes from *Vibrio harveyi*and expression of bioluminescence in *Escherichia coli*. Biochemistry, 23: 3663-3667.
63. Koncz C, Olsson O, Langridge WH, Schell J, Szalay AA (1987) Expression and assembly of functional bacterial luciferase in plants. Proceedings of theNational Academy of Sciences USA, 84: 131-135.
64. Langridge WHR, Fitzgerald KJ, Koncz C, Schell J, Szalay AA (1989) Dual promoter of Agrobacteriumtumefaciensmannopine synthase genes is regulated by plant growth hormones. Proceedings of the National Academy ofSciences USA, 86: 3219-3223.
65. Langridge W, Escher A, Wang G, Ayre B, Fodor I, Szalay A (1994) Lowlight image analysis of transgenic organisms using bacterial luciferase as a marker. Journal of Bioluminescence and Chemiluminescence, 9: 185-200.
66. Johansson AM, Wang C, Stenberg A, Hertzberg M, Little CH, Olsson O (2003) Characterization of a PttRPS18 promoter active in the vascular cambium region of hybrid aspen. Plant Molecular Biology, 52: 317-329.
67. Almond B, Hawkins E, Stecha P, Garvin D, Paguio A, Butler B, Beck M,Wood M, Wood K (2003) A new luminescence: Not your average click beetle. Promega Notes, 85: 11-14.
68. Illarionov, B. A., V. M. Blinov, A. P. Donchenko, M. V. Protopopova, V. A. Karginov, N. P. Mertvetsov, and J. I. Gitelson, 1990. Isolation of bioluminescent functions from *Photobacteriumleiognathi*: analysis of*lux*A, *lux*B, *lux*G and neighboring genes. Gene, 86: 89-94.

69. Baldwin, T. O., J. H. Devine, R. C. Heckel, J.-W. Lin, and G. S. Shadel. 1989. The complete nucleotide sequence of the *lux*regulon of *Vibrio fischeri* and the *lux*ABN region of *Photobacteriumleiognathi* and the mechanism of control of bacterial bioluminescence. J. Biolumin. Chemilumin, 4: 326-341.
70. Soly, R. R., J. A. Mancini, S. R. Ferri, M. Boylan, and E. A. Meighen. 1988. A new *lux* gene in bioluminescent bacteria codes for a protein homologous to the bacterial luciferase subunits. Biochem. Biophys. Res. Commun, 155: 351-358.
71. Lee, C. Y., and R. Szittner. Unpublished data.
72. Engebrecht, J., K. H. Nealson, and M. Silverman. 1983. Bacterial bioluminescence: isolation and genetic analysis of functions from *Vibrio fischeri*. Cell, 32: 773-781.
73. Engebrecht, J., and M. Silverman. 1984. Identification of genes and gene products necessary for bacterial bioluminescence. Proc. Nati. Acad. Sci. USA, 81: 4154-4158.
74. Engebrecht, J., and M. Silverman. 1986. Regulation of expression of bacterial genes for bioluminescence. Genet. Eng., 8: 31-44.
75. Swartzman, E., S. Kapoor, A. F. Graham, and E. A. Meighen. 1990. A new *Vibrio fischerilux* gene precedes a bidirectional termination site for the *lux* operon. J. Bacteriol, 172: 6797-6802.
76. Swartzman, E., C. Miyamoto, A. Graham, and E. Meighen. 1990. Delineation of the transcriptional boundaries of the *lux* operon of *Vibrio harveyi* demonstrates the presence of two new *lux* genes. J. Biol. Chem., 265: 3513-3517.
77. Martin, M., R. Showalter, and M. Silverman. 1989. Identification of a locus controlling expression of luminescence genes in *Vibrio harveyi*. J. Bacteriol, 171: 2406-2414.
78. Meighen, E. A., and R. E. MacKenzie. 1973. Flavine specificity of enzyme-substrate intermediates in the bacterial bioluminescent reaction. Biochemistry, 12: 1482-1491.
79. Meighen, E. A., and G. G. Grant. 1985. Bioluminescence analysis of long chain aldehydes: detection of insect pheromones, p. 253-268. In K. Van Dyke (ed.), Bioluminescence and chemiluminescence: instruments and applications, vol. II. CRC Press, Inc., Boca Raton, Fla.
80. Meighen, E. A., K. N. Slessor, and G. G. Grant. 1982. Development of a bioluminescence assays for aldehyde pheromones of insects. I. Sensitivity and specificity. J. Chem. Ecol, 8: 911-921.
81. Rodriguez, A., I. R. Nabi, and E. Meighen. 1985. ATP turnover by the fatty acid reductase complex of *Photobacteriumphosphoreum*. Can. J. Biochem. Cell Biol, 63: 1106-1111.

82. Shimomura, O., F. H. Johnson, and H. Morise. 1974. The aldehyde content of luminous bacteria and of an "aldehydeless" dark mutant. Proc. Natl. Acad. Sci. USA, 71: 4666-4669.
83. Ulitzur, S., and J. W. Hastings. 1979. Evidence for tetradecanal as the natural aldehyde in bacterial bioluminescence. Proc. Natl. Acad. Sci. USA, 76: 265-267.
84. Colepicolo, P., K.-W.Cho, G. 0.Poinar, and J. Hastings. 1989. Growth and luminescence of the bacterium *Xenorhabdusluminescens* from a human wound. Appl. Environ. Microbiol, 55: 2601-2606.
85. Meighen, E. A., and I. Bartlet. 1980. Complementation of subunits from different bacterial luciferases. Evidence for the role of the P subunit in the bioluminescent mechanism. J. Biol.Chem., 255: 11181-11187.
86. Schmidt, T. M., K. Kopecky, and K. H. Nealson. 1989. Bioluminescence of the insect pathogen *Xenorhabdusluminescens*. Appl. Environ. Microbiol, 55: 2607-2612.
87. Szittner, R., and E. Meighen. 1990. Nucleotide sequence, expression and properties of luciferase coded by *lux* genes from a terrestrial bacterium. J. Biol. Chem., 265: 16581-16587.
88. Hastings, J. W., C. J. Potrikas, S. C. Gupta, M. Kurfurst, and J. C. Makemson. 1985. Biochemistry and physiology of bioluminescent bacteria. Adv. Microbiol. Physiol., 26: 235-291.
89. Lee, J. 1985. The mechanism of bacterial bioluminescence, p. 401-437.In J. G. Burr Chemiluminescence and bioluminescence.Marcel Dekker, Inc., New York.
90. Baldwin, T. O., M. M. Ziegler, and D. A. Powers. 1979. Covalent structure of subunits of bacterial luciferase: NH2- terminal sequence demonstrates subunit homology. Proc. Natl. Acad. Sci. USA, 76: 4887-4889.
91. Baldwin, T. O., L. H. Chen, L. J. Chlumsky, J. H. Devine, T. C. Johnston, J. W. Lin, J. Sugihara, J. J. Waddle, and M. M. Ziegler. 1987. Structural analysis of bacterial luciferase, p. 621-631. In E. E. Edmondson and D. B. McCormick (ed.), Flavins and flavoproteins. Walter de Gruyter & Co., Berlin.
92. Haygood, M. G. 1990. Relationship of the luminous bacterial symbiont of the Caribbean flashlight fish, Kryptophanaronalfredi (family Anomalopidae), to other luminous bacteria based on bacterial luciferase *(lux*A) genes. Arch. Microbiol, 154: 496-503.
93. Riendeau, D., A. Rodriguez, and E. A. Meighen. 1982. Resolution of the fatty acid reductase from *Photobacteriumphos- phoreum* into acyl-protein synthetase and acyl-

CoA reductase activities. Evidence for an enzyme complex. J. Biol. Chem., 257: 6908-6915.

94. Rodriguez, A., D. Riendeau, and E. Meighen. 1983. Purification of the acyl-CoA reductase component from a complex responsible for the reduction of fatty acids in bioluminescent bacteria. Properties and acyl-transferase activity. J. Biol. Chem., 258: 5233-5238.
95. Rodriguez, A., L. Wall, D. Riendeau, and E. A. Meighen. 1983. Fatty acid acylation of proteins in bioluminescent bacteria. Biochemistry, 22: 5604-5611.
96. Boylan, M., A. F. Graham, and E. A. Meighen. 1985. Functional identification of the fatty acid reductase components encoded in the luminescence operon of *Vibrio fischeri*. J.Bacteriol, 163: 1186-1190.
97. Boylan, M., C. Miyamoto, L. Wall, A. F. Graham, and E. A. Meighen. 1989. *Lux*C, D and E genes of the *V. fischeri* luminescence operon code for the reductase, transferase and synthetase enzymes involved in aldehyde biosynthesis. Photochem. Photobiol, 49: 681-688.
98. Engebrecht, J., and M. Silverman. 1984. Identification of genes and gene products necessary for bacterial bioluminescence. Proc. Nati. Acad. Sci. USA, 81: 4154-4158.
99. Meighen, E. A. 1988. Enzymes and genes from the *lux* operons of bioluminescent bacteria.Ann. Rev. Microbiol, 42: 151-176.
100. Byers, D. M., and E. A. Meighen. 1985. Acyl-acyl carrier protein as a source of fatty acids for bacterial bioluminescence. Proc. Natl. Acad. Sci. USA, 82: 6085-6089.
101. Wall, L., A. Rodriguez, and E. Meighen. 1986. Intersubunit transfer of fatty acyl groups during fatty acid reduction. J. Biol. Chem, 261: 16018-16025.
102. Wall, L. A., and E. A. Meighen. 1986. Subunit structure of the fatty acid reductase complex from *Photobacteriumphosphoreum*. Biochemistry, 25: 4315-4321.
103. O'Kane, D. J., J. Vervoort, F. Muller, and J. Lee. 1987. Purification and characterization of an unusual non-fluorescent flavoprotein from *Photobacteriumleiognathi*, p. 641-645. In D. E. Edmondson and B. B. McCormick (ed.), Flavins and flavoproteins. Walter de Gruyer & Co., Berlin.
104. Kasai, S., S. Fujii, R. Miura, S. Odani, T. Nakaya, and K. Matsui. In Flavins and flavoproteins, in press.
105. Soly, R. R., J. A. Mancini, S. R. Ferri, M. Boylan, and E. A. Meighen. 1988. A new *lux* gene in bioluminescent bacteria codes for a protein homologous to the bacterial luciferase subunits. Biochem.Biophys. Res. Commun, 155: 351-358.

106. Swartzman, E., S. Kapoor, A. F. Graham, and E. A. Meighen. 1990. A new *Vibrio fischerilux* gene precedes a bidirectional termination site for the *lux* operon. J. Bacteriol, 172: 6797-6802.
107. Swartzman, E., C. Miyamoto, A. Graham, and E. Meighen. 1990. Delineation of the transcriptional boundaries of the *lux* operon of *Vibrio harveyi* demonstrates the presence of two new *lux* genes. J. Biol. Chem, 265: 3513-3517.
108. Showalter, R. E., M. 0. Martin, and M. R. Silverman. 1990. Cloning and nucleotide sequence of *lux*R, a regulatory gene controlling bioluminescence in *Vibrio harveyi*. J. Bacteriol, 172: 2946-2954.
109. Engebrecht, J., K. H. Nealson, and M. Silverman. 1983. Bacterial Bioluminescence: isolation and genetic analysis of functions from *Vibrio fischeri*. Cell, 32: 773-781.
110. Ruby, E.-G., and K. H. Nealson. 1977. A luminous protein that emits yellow light. Science, 196: 432-434.
111. Eckstein, J. W., K. W. Cho, P. Colepicolo, S. Ghisla, and J. W. Hastings, and T. Wilson. 1990. A time-dependent bacterial bioluminescence emission spectrum in an in vitro single turnover system: energy transfer alone cannot account for the yellow emission of *Vibrio fischeri* Y-1. Proc. Natl. Acad. Sci. USA, 87: 1466-1470.
112. Byers, D., and E. A. Meighen. 1984. *Vibrio harveyi* aldehyde dehydrogenase. Partial reversal of aldehyde oxidation and its possible role in the reduction of fatty acids for the bioluminescence reaction. J. Biol. Chem, 259: 7109-7114.
113. Byers, D. M., A. Bognar, and E. A. Meighen, 1988. Differential regulation of enzyme activities involved in aldehyde metabolism in the luminescent bacterium *Vibrio harveyi*. J. Bacteriol, 170: 967-971.
114. Ziegler MM, Baldwin TO (1981): Biochemistry of bacterial bioluminescence. Curr Top Bioenerg, 12: 65-113.
115. Hastings WJ, NealsonKH (1977): Bacterial bioluminescence. Annu Rev Microbiol, 31: 549-595.
116. Hastings WJ (1978): Bacterial bioluminescence: An overview. In De-Luca M (ed): "Bioluminescence and Chemiluminescence." Methods Enzymol, 57: 125-135.
117. Kurfiirst M, Ghisla S, Hastings W (1984): Characterization and postulated structure of the primary emitter in the bacterial luciferase reaction. ProcNatlAcadSci USA, 81: 2990-2994.

118. Aboukhair NK, Ziegler MM, Baldwin TO (1985): Bacterial luciferase: Demonstration of a catalyticall-y com-p etent altered conformational state follow?ng a single turnover. Biochemistry, 24: 3942-3947.
119. Paquette 0, Fried A, TuSC (1988): Delineation of bacterial luciferase aldehyde site by bifunctional labeling reagents. Arch Biochem Bicphys, 264: 392-399.
120. Chen LH, Baldwin TO (1989): Random and site-directed mutagenesis of bacterial luciferase: Investigation of the aldehyde binding site. Biochemistry, 28: 2684-2689.
121. Lee J, O'Kane DJ, Gibson BG (1989): Bioluminescence spectral and fluorescence dynamics study of the interaction of lumazine protein with the intermediates of bacterial luciferase bioluminescence. Biochemistry, 28: 4263-4271.
122. VanDyke K (1985): "Bioluminescence and Chemiluminescence: Instruments and Applications." Boca Raton, Florida: CRC Press.
123. Weinhausen G, DeLuca M (1982): Bioluminescent assays of picomole levels of various metabolites using immobilized enzymes. Anal Biochem, 127: 380-388.
124. Campbell AK, Hallett MB, Weeks I (1985): Chemiluminescence as an analytical tool in cell biology and medicine. In Glick D (ed): Methods in Biochem Anal, 31: 317-416.
125. Ulitzur S (1986): Determination of antibiotic activities with the aid of luminous bacteria. In DeLuca M, McElroy WD (eds): "Bioluminescence and Chemiluminescence." Methods Enzymol, 133: 275-284.
126. KrickaLJ (1988): Clinical and biochemical applications of luciferases and luciferins. Anal Biochem, 175: 14-21.
127. Baldwin TO, Holzman TF, Holzman R (1986): Active center-based immuno-assay approach using bacterial luciferase. In DeLuca M, McElroy WD (eds): "Bioluminescence and Chemiluminescence." Methods Enzymol, 133: 248-264.
128. Haubner R, Geiger R (1988): A sensitive, bioluminescent enhanced detection method for DNA dot-hybridization. Nucleic Acids Res, 16: 1213.