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Thoughts on the diversity of convergent evolution of bioluminescence on earth

Hans E. Waldenmaier[1], Anderson G. Oliveira[2] and Cassius V. Stevani[1]

[1]*Departamento de Química Fundamental, Instituto de Química, Universidade de São Paulo, CP 26077, 05599-970 São Paulo, SP, Brazil*
[2]*Departamento de Genética e Evolução, Universidade Federal de São Carlos, 13500-900, Sorocaba, SP, Brazil*
e-mail: stevani@iq.usp.br

Abstract: The widespread independent evolution of analogous bioluminescent systems is one of the most impressive and diverse examples of convergent evolution on earth. There are roughly 30 extant bioluminescent systems that have evolved independently on Earth, with each system likely having unique enzymes responsible for catalysing the bioluminescent reaction. Bioluminescence is a chemical reaction involving a luciferin molecule and a luciferase or photoprotein that results in the emission of light. Some independent systems utilize the same luciferin, such as the use of tetrapyrrolic compounds by krill and dinoflagellates, and the wide use of coelenterazine by marine organisms, while the enzymes involved are unique. One common thread among all the different bioluminescent systems is the requirement of molecular oxygen. Bioluminescence is found in most forms of life, especially marine organisms.

Bioluminescence in known to benefit the organism by: attraction, repulsion, communication, camouflage, and illumination. The marine ecosystem is significantly affected by bioluminescence, the only light found in the pelagic zone and below is from bioluminescent organisms.

Transgenic bioluminescent organisms have revolutionized molecular research, medicine and the biotechnology industry. The use of bioluminescence in studying molecular pathways and disease allows for non-invasive and real-time analysis. Bioluminescence-based assays have been developed for several analytes by coupling luminescence to many enzyme-catalysed reactions.

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Introduction

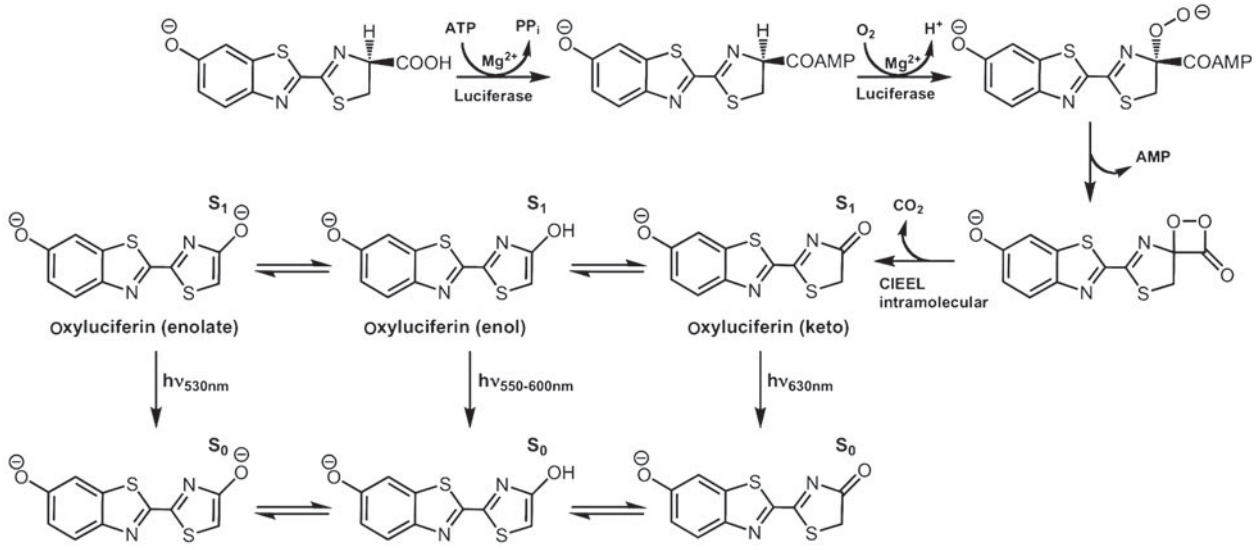
One of the most impressive and diverse forms of convergent evolution on Earth is bioluminescence: the generation of light from a chemical reaction in a living organism. Almost every domain of life has some bioluminescent species, with notable exceptions including plants, birds, reptiles and mammals. Terrestrial bioluminescent species include arthropods, bacteria, earthworms and fungi. The majority of bioluminescent species are aquatic marine organisms. There are over 30 different independent bioluminescent systems that have evolved in extant species, each system likely having unique enzymes responsible for catalysing the bioluminescent reaction (Wilson & Hastings 1998). One common thread among all the different bioluminescent systems is the requirement of molecular oxygen.

Convergent evolution is the independent development of the same biological trait in unrelated lineages. These traits are said to be analogous to each other as opposed to homologous, which involves a common ancestor. Some classic examples of convergent evolution are the wings of bats and birds; bipedal locomotion of apes, birds and some octopuses; and, opposable thumbs in primates and opossums. There are many other less obvious examples of convergent evolution that occur at the molecular level. Both fungi and plants produce and use gibberellin and abscisic acid hormones (Siewers *et al.* 2004; Tudzynski 2005). The gibberellin made by plants and fungi is structurally identical; the biosynthetic pathway is highly divergent between the two lineages. The same is true for abscisic acid production in plants and fungi. Both bats and dolphins use echolocation to detect objects in their environment; a critical component of this trait is the ability to listen to the echoes returning from the objects. The gene *Prestin* is partially responsible for cochlear amplification, when the sequences of the *Prestin* gene are compared among some mammals, the bat and dolphin genes cluster together, both species have amino-acid substitutions in similar locations (Liu *et al.* 2010). Given the diversity and quantity of bioluminescent organisms, little attention has been given to its convergent evolution.

In this work, we will examine bioluminescence from three perspectives: (i) the bioluminescent species on Earth will be introduced, and species will be grouped together by luciferin substrate; (ii) the ecological significance will be discussed and (iii) an overview of the biotechnological applications of bioluminescence will be presented.

The luciferins of bioluminescent organisms

A luciferin is a small molecule that generates light upon a chemical reaction with oxygen catalysed by an enzyme



Scheme 1. Reaction mechanism of firefly bioluminescence.

generically called luciferase (Wilson & Hastings 1998; Shimomura 2006). Other luciferins interact with photoproteins. One of the main differences between luciferases and photoproteins is the dependence of oxygen in cell-free luminescent reactions, photoproteins do not require oxygen for *in vitro* luminescence although oxygen is required for photoprotein synthesis, the net luminescence is directly correlated with the protein amount, as the photoprotein acts as reactant in the reaction being consumed (Shimomura 2006). One can also consider photoproteins a subset of luciferases with low turnover, in which the luciferin binds covalently with the protein. Luciferases require oxygen in the cell-free luminescence reaction and they act as a catalyst (enzyme) and can be recycled. The total amount of light is dependent on the quantity of luciferin substrate. At the chemical level, most bioluminescent light is generated as a result of the decomposition of a four-membered ring dioxetanone (Wilson & Hastings 1998). These strained and energy-rich peroxides require low energy to break, but their decomposition yields a molecule in the electronically excited state, whose decay to the ground state is accompanied by light emission. Some bioluminescent systems do not employ a dioxetanone intermediate, but an acyclic peroxide (a hemiperoxyacetal), such as bacterial systems. Although there are over 30 bioluminescent systems (Wilson & Hastings 1998; Shimomura 2006), there are only nine well-defined luciferins. A luciferin and its luciferase are usually encountered only within a single lineage. The exception includes some coelenterazine-dependent bioluminescent organisms from Phyla: Sarcomastigophora (protozoa), Cnidaria, Ctenophora, Mollusca, Arthropoda and Chordata (pisces), despite their common use of coelenterazine the luciferases differ or are currently unknown (Wilson & Hastings 1998; Szent-Gvorgvi *et al.* 1999).

Firefly luciferin

Depending on firefly species, the *in vivo* light emission peaks range from 552 to 582 nm (Seliger & McElroy 1964). Structures of firefly luciferin and firefly luciferase have been determined (White *et al.* 1961; Conti *et al.* 1996). cDNA clones of firefly luciferase have been isolated and translated *in vitro* (Wood *et al.* 1984). Firefly luciferase catalyses the reaction of firefly luciferin with ATP and molecular oxygen, resulting in light emission (from green until red), CO_2, AMP and oxyluciferin. The mechanism (Scheme 1) involves the formation of a dioxetanone ring common to other bioluminescent systems (Shimomura *et al.* 1977). There are three families of Coleoptera that are bioluminescent, Lampyridae (fireflies), Phengodidae (railroad worms) and Elateridae (click beetles). All three share the same basic bioluminescent mechanism and catalyse the same luciferin as the well-studied lampirids, although species of Phengodidae and Elateridae have homologous luciferases that emit different colours (Wood *et al.* 1989; Viviani *et al.* 1999).

Some Diptera emit blue bioluminescence, the mechanism is poorly understood, lacking structures for luciferin and luciferase, and a detailed reaction mechanism. Diptera bioluminescence is likely independent of Coleoptera bioluminescence. The Diptera *Arachnocampa luminosa* and *Orfelia fultoni* appear to have different bioluminescent systems based on the specificity of different cofactors. Each system is likely to have unique luciferin and luciferase structures (Viviani 2002; Shimomura 2006).

Tetrapyrrole compound luciferins: Dinoflagellates and Krill

Krill and dinoflagellates luminesce at 476 and 474 nm, respectively (Nakamura *et al.* 1988; Nakamura *et al.* 1989), and both bioluminescent systems use very similar tetrapyrrolic compound as luciferin, differing only by a hydroxyl group moiety. Regarding the luciferases, they are analogous, showing a noteworthy example of convergent evolution in bioluminescence. In fact, the chemical pathways involved are the same, and luminescence was observed in a cross-reaction between krill luciferin and dinoflagellate luciferase, and vice-versa (Dunlap *et al.* 1980). The krill species *Euphoausia pacifica* and *Meganyctiphanes norvegica* are the most studied

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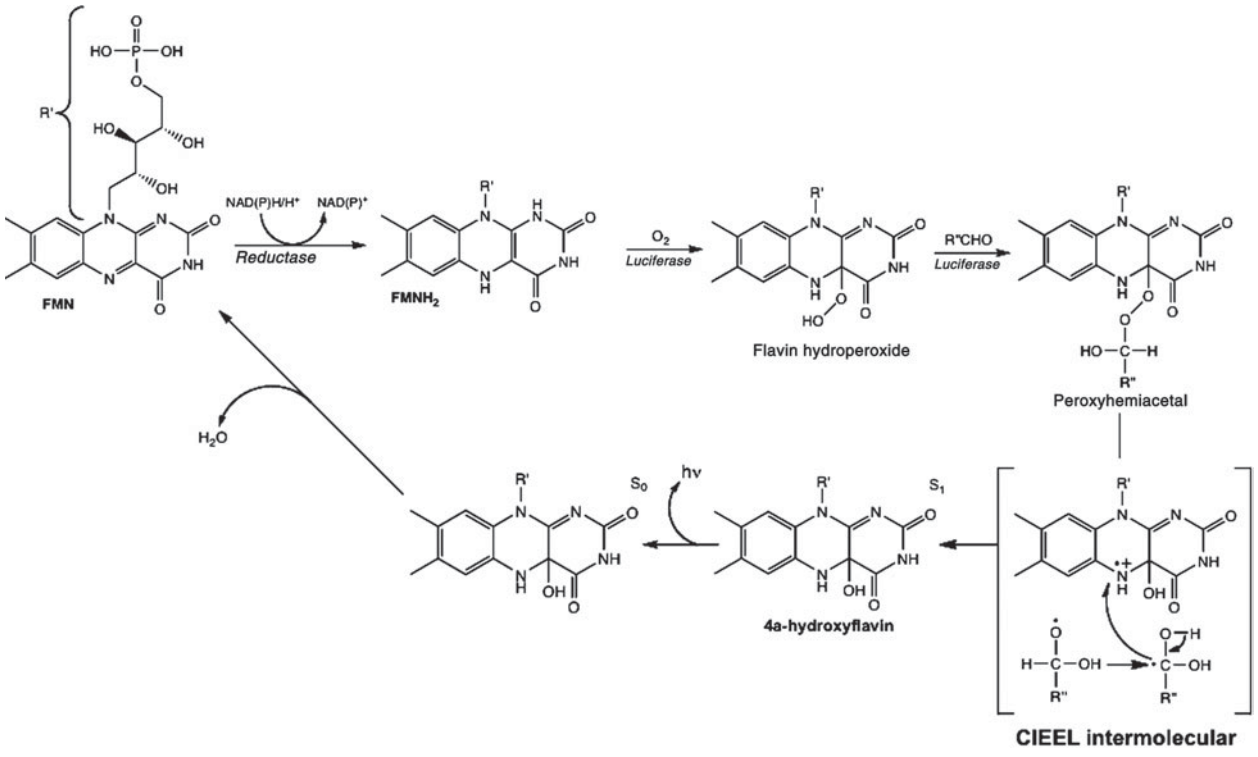

Scheme 2. Reaction mechanism of bacterial bioluminescence.

bioluminescent Euphausiids, and both species are widely distributed in marine environments (Shimomura 2006). Nakamura *et al.* (1988) proposed a structure for the luciferin and Shimomura (1995) characterized the purified luciferase.

The dinoflagellate system is better understood than krill. Dinoflagellate blooms are responsible for red tide 'phosphorescence' in the sea (Wilson & Hastings 1998). The structure of dinoflagellate luciferin was determined by Nakamura *et al.* (1989), and the bioluminescent pathway does not involve a dioxetanone intermediate (Stojanovic & Kishi 1994). The gene responsible was cloned (Bae & Hastings 1994), and the crystal structure for functional 35 kDa dinoflagellate luciferase was solved by Schultz *et al.* (2005).

Cypridina *luciferin*

Cypridina hilgendorfii has been used as a tool to understand the biochemistry of bioluminescence in the 20th century. When disturbed it secretes a luminous mucus (light in the range of 450–460 nm) from a specialized gland with two types of cells, one cell type secreting luciferin and the other secreting luciferase, resulting in a bright blue cloud of bioluminescence (Shimomura & Johnson 1970; Shimomura 2006). The structure of the luciferin was determined by Kishi *et al.* (1966) and the luciferase was cloned by Thompson *et al.* (1989). Shimomura & Johnson (1971) demonstrated the involvement of a dioxetanone intermediate in the luminescence reaction mechanism. The luciferase of *Pyrocypris noctiluca*, in the same class as *C. hilgendorfii*, has also been cloned, and the two homologous proteins have 83.1% sequence identity (Nakajima *et al.* 2004). Moreover, several coastal luminous fish also utilize *Cypridina* luciferin although their luciferases are likely analogous to *C. hilgendorfii*.

Odontosyllis *luciferin*

The Bermuda fireworm *Odontosyllis* is one of the least understood bioluminescence systems. *Odontosyllis* luminescence is correlated with the lunar cycle, for 7 days following a full moon the Bermuda Fireworm lumineses at sunset (Morin 1983). Harvey (1952) demonstrated a luciferin–luciferase reaction using whole-worm extracts. However, the luminous mucus (light emission 507 nm) of the fireworm may emit light from a secreted photoprotein, as the classical luciferin–luciferase assay did not produce luminescence, and the reaction still occurs in the absence of oxygen suggesting that *Odontosyllis* may have two separate luminescence mechanisms, as suggested by Dehyan & Latz (2009).

Diplocardia *luciferin*

Some earthworms, the majority in the Megascolecidae family, bioluminesce in the range of 490–507 nm (Bellisario *et al.* 1972; Ohtsuka *et al.* 1976). Some earthworms secrete luminous mucus from their mouth, anus and pores. The most studied organism is *Diplocardia longa*, from which a substance has been isolated and proposed as luciferin (Ohtsuka *et al.* 1976), and the luciferase activity evidenced (Rudie *et al.* 1981). Light emission could be obtained using a cell-free extract upon addition of hydrogen peroxide. Moreover, a flavin has been

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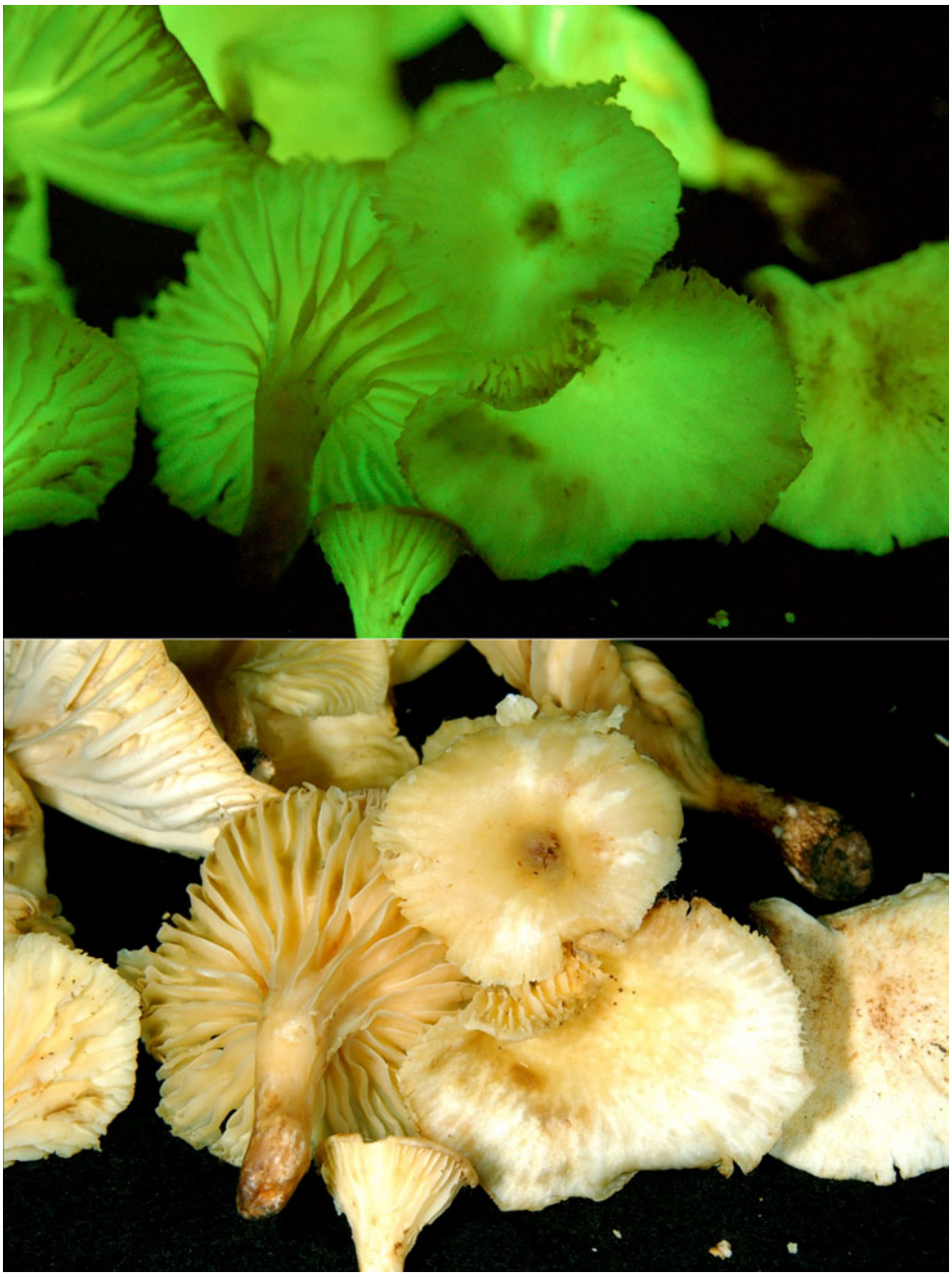

Fig. 1. Bioluminescence of *Neonothopanus gardneri* photographed in dark conditions (above) and in the light (below).

suggested to be involved in the luminescent reaction (Shimomura 2006), but this still requires further confirmation.

Latia *luciferin*

The limpet *Latia neritoides* emits light at 536 nm (Shimomura & Johnson 1968b) and is the only known freshwater luminescent organism, apart from some bacteria and larval stages of fireflies (Shimomura 2006). Latia luminescence is extracellular and is due to the secretion of luciferin and luciferase, which were shown to participate in the process by the hot and cold luciferase assay (Bowden 1950). A putative structure of the fat soluble Latia luciferin was proposed by Shimomura & Johnson (1968a). The luminescence reaction requires luciferin, luciferase, molecular oxygen and a 'purple protein' of unknown function (Shimomura & Johnson 1968b; Kojima *et al.* 2000). Later on, Kojima showed that the purple protein is not necessary to obtain light emission (Kojima *et al.* 2000). The reaction mechanism is not known, but is known to form formic acid as a product (Shimomura & Johnson 1968b).

Bacterial luciferin

Bacteria emit light at 490 nm from the decomposition of the peroxyhemiacetal formed by the reaction among reduced

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flavin mononucleotide ($FMNH_2$), oxygen and a long-chain aldehyde inside the active site of the luciferase (Cormier & Strehler 1953). Several species of bacteria are known to produce light including the well-studied *Vibrio harveyi* and *Vibrio fischeri*. The *lux* operon contains genes for bacteria luciferase, *luxA* and *luxB*, genes for the fatty-acid luciferin reductase complex, *luxC, lucD* and *luxE* (Meighen 1991). Moreover, species-specific bioluminescence genes are also found in the *lux* operon (Meighen 1994). Depending on the species, several flavin-reductases yield $FMNH_2$ a common one being homologous to the *E. coli* flavin reductase *Fre* (Zenno & Saigo 1994). As already mentioned, the reaction (Scheme 2) does not produce a dioxetanone intermediate, but instead an FMN hydroperoxide, which on its turn adds to a long-chain aldehyde producing a hemiperoxyacetal, whose decomposition via the so-called Chemically Initiated Electron Exchange Luminescence (CIEEL) leads to the formation of hydroxy-FMN in the electronically excited state (Eckstein *et al.* 1993; Villa & Willetts 1997; Stevani & Baader 1999; Berkel *et al.* 2006). The bacterial luciferase and the NADH/NADPH-dependent flavin-reductase form a complex as demonstrated by affinity studies (Watanabe & Hastings 1982) and kinetic experiments (Tu 2007).

Fungal luciferin

The structure of the fungal luciferin is currently unknown (Oliveira & Stevani 2009). There are no less than 71 species of luminescent fungi, representing four distinct lineages belonging to the Omphalotaceae, Armillaria, Mycenaceae and Lucentipes lineages (Desjardin *et al.* 2008; Desjardin *et al.* 2010; Oliveira *et al.* 2012). Fungal bioluminescence is poorly understood, but ongoing work performed by our group could answer some questions. Desjardin *et al.* (2008) wrote the most current and comprehensive review on this subject. The spatial distribution of bioluminescence varies among species. In some species the whole fruiting body emits light (Fig. 1), and in others only some part of the fruiting body is luminescent (*i.e.*, stipe or pileus), while the mycelium is non-luminescent or vice-versa (Desjardin *et al.* 2008). In 1959, Airth and McElroy were the first to successfully perform the classical luciferin/luciferase test in fungi (Airth & McElroy 1959). The mixture of the luciferin-rich hot extract, enzyme-rich cold extract and NADH or NADPH was required for sustained *in vitro* light emission (Airth & McElroy 1959). The cold water extract can be further separated by ultracentrifugation into soluble (supernatant) and insoluble (pellet) fractions each containing essential enzymes for the luminescent reaction and suggesting a two-step enzymatic reaction (Scheme 3) very similar to the bacterial mechanism. The first step involves a dark reaction with the supernatant containing the NAD(P)H-reductase and exogenous NAD(P)H, this occurs prior to the light generating step, which is catalysed by the luciferase-rich insoluble fraction (Airth & Foerster 1962). In 2009, the enzymatic nature of fungal bioluminescence was finally confirmed using hot and cold extracts prepared from luminescent fungi and NADPH (Oliveira & Stevani 2009). Moreover, the authors were also able to match the *in vivo* bioluminescence spectrum to the *in vitro* one obtained from hot and cold extracts, further supporting that the light emission processes were the same. Preliminary results indicate that the luciferin may have a flavin-like structure. However, the addition of FMN in the presence or absence of a long-chain aldehyde did not lead to light emission (unpublished data).

Fungal bioluminescence
- Proposal from Airth & Foerster

$$L + NAD(P)H + H^+ \xrightarrow[(reductase)]{\text{soluble enzyme}} LH_2 + NAD(P)^+$$

$$LH_2 + O_2 \xrightarrow[(luciferase)]{\text{insoluble enzyme}} LO + H_2O + h\nu$$

L: luciferin, LH_2: reduced luciferin, LO: oxyluciferin

Bacterial bioluminescence

$$FMN + NAD(P)H + H^+ \xrightarrow{reductase} FMNH_2 + NAD(P)^+$$

$$FMNH_2 + RCHO + O_2 \xrightarrow{luciferase} FMN + RCO_2H + H_2O + h\nu$$

FMN: oxidized flavin mononucleotide (luciferin), $FMNH_2$: reduced FMN
RCHO: long-chain aldehyde RCO_2H: fatty acid

Scheme 3. Proposed mechanism of fungal bioluminescence.

The luciferin Coelenterazine

Coelenterazine is used as luciferin by many analogous systems, such as the protozoan *Thalassicollin* and six other animal phyla: Athropoda, Cnidaria, Mollusca, Ctenophora, Chordata and Echinodermata (Campbell & Herring 1990; Shimomura 2006). All bioluminescent species that use coelenterazine are marine organisms. Notwithstanding the luciferin molecule is the same for these systems, the enzymes that catalyse the luminescent reaction (Scheme 4) are similar, but of independent origin. Only some species synthesize their own coelenterazine the rest relying on dietary sources, as there are many non-luminous species that contain large quantities of coelenterazine. Coelenterazine can be used by the organism either with a luciferase or with a photoprotein. In all cases the light emission is triggered by Ca^{2+}.

Functions of bioluminescence

Bioluminescence should serve to some beneficial purpose to the organism otherwise it would have been selected against and not evolved so many times. The significance of bioluminescence must be species specific, as the behaviours and characteristics of these organisms are diverse. The ecological significance of bioluminescence in fish is likely different from that for fungal bioluminescence, the fish being a mobile marine animal that can see light, the other a stationary decomposer that has no nervous system. There are two main environments where bioluminescence occurs, on the ground and in the ocean. There are significant differences in how light affects the marine environment than on land (Widder 2001). The epipelagic zone of the ocean is illuminated by sunlight during the day and is dark by night. The only illumination the mesopelagic zone receives is dim light from above. The pelagic zone and below is only illuminated by bioluminescence (Widder 2001). There is

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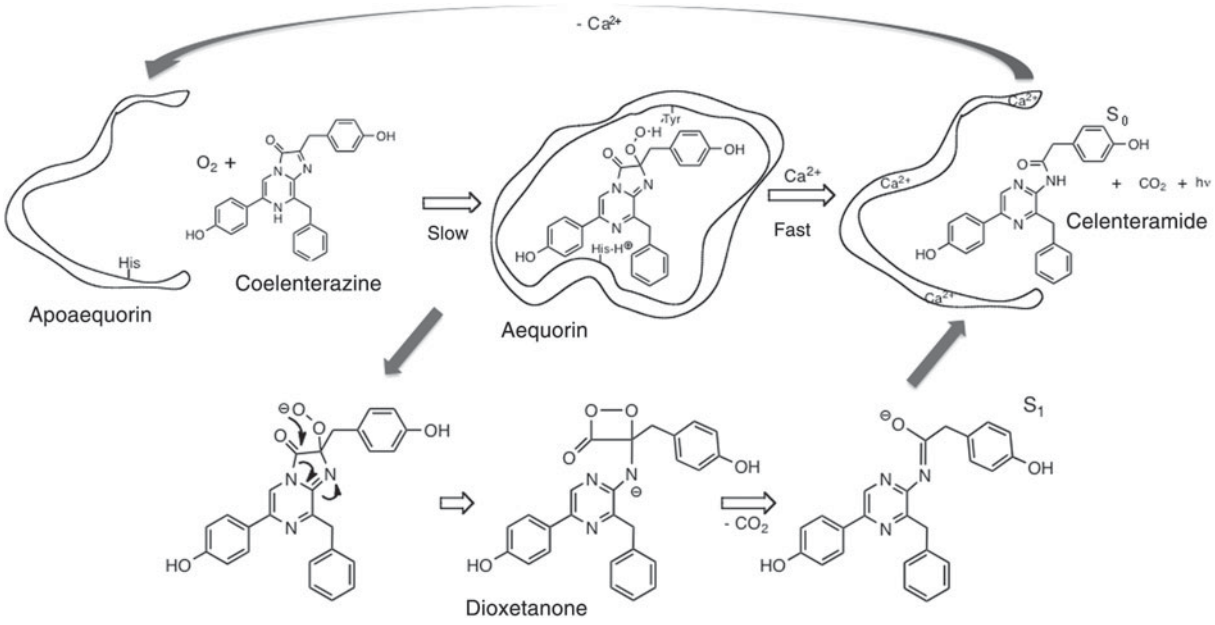

Scheme 4. Reaction mechanism of bioluminescence in a coelenterazine-based photoprotein system.

no barrier between these zones and many marine organisms hide in the mesopelagic or pelagic zones during the day and migrate to the surface epipelagic zone at night to feed closer to the surface. In the open-ocean there is no place to hide, everything is exposed (Widder 2001). Most marine bioluminescence light is in the blue region, which closely matches the wavelengths of maximum transmission through seawater, this is also similar to the wavelength of maximal sensitivity for most pelagic fish and crustaceans. When monitoring basal bioluminescence levels in the ocean it was observed that mechanical disturbance by equipment and animals was the main trigger of plankton bioluminescence, any animal seeking darkness as a mean of evading detection must cope with a potential bioluminescent minefield (Widder 2001). Bioluminescence could be thought to function in one of several ways, attraction, repulsion, camouflage, illumination and communication (Sivinski 1981; Sivinski 1998). These are likely to occur on land and the sea.

Larva of luminescent Diptera Mycetophilidae use their bioluminescence to lure phototropic prey into bead-shaped webs, also disturbance of luminescent Diptera larvae or pupae changes their light emission and may be acting as a warning signal to negatively phototropic predators (Gatenby 1959; Sivinski 1998). Dinoflagellates luminesce in response to mechanical disturbance. When a dinoflagellate grazer disturbs its prey the cloud of light then attracts the predator of the dinoflagellate grazer. This has been verified experimentally by monitoring numbers of copepods (grazers of dinoflagellates) and sticklebacks (predators of copepods) in aquariums with luminescent or non-luminescent dinoflagellates. In tanks with luminescent dinoflagellates, copepod numbers were lower (Abrahams & Townsend 1993). Attraction of mates has been observed on the land and in the sea, with fireflies and ostracods, respectively.

Bioluminescence could also benefit the individual by repelling unwanted pests or predators or tricking them into staying away. Caribbean ostracods can release large clouds of bioluminescence that luminesces for several seconds, which can blind or confuse a predator (Widder 2001). Repulsion of fungivores is a proposed function of fungal bioluminescence (Sivinski 1981).

As mentioned, the mesopelagic zone of the ocean only receives very dim sunlight from above. Frequently, organisms have downward facing bioluminescent organs presumably to disrupt their silhouette to predators or prey below (Widder 2001). Some species of fish use luminescence to illuminate the water directly in front of them, such as the Stomiiformes fish that have far-red bioluminescent (> 700 nm) light organs below their eyes and photosensors inside them, with the rare ability to detect this wavelength of light (Sutton 2005; Kenaley 2010). It is thought that this top predator of the mesopelagic zone has evolved the ability to illuminate and see its prey.

Applications of bioluminescence

Since the late 1980s bioluminescent transgenic organisms have been engineered, such as bioluminescent tobacco plants transformed with the firefly *luc* gene (Ow *et al.* 1986). These plants did not promptly bioluminesce, they had to be soaked in a firefly luciferin solution and long exposure photography was required to see the luminescence. Nevertheless, engineered bioluminescent organisms offer new and useful applications (Roda *et al.* 2004). Some of the commonly used luminescent genes are the bacterial *lux* operon, firefly *luc* and *aequorin* genes. In some compatible organisms, the *lux* operon requires no exogenous factors for expression, just $FADH_2$ and the long-chain aldehyde, which can be found in some organisms (Francis *et al.* 2000). The firefly luciferase and the *aequorin*

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photoprotein require additional exogenous substrates such as firefly luciferin and coelenterazine, respectively. The fluorescent, green fluorescent protein (GFP), found in a bioluminescent jellyfish is often used in research applications. GFP was discovered along with the *Aequorea victoria* luminescent photoprotein aequorin (Shimomura *et al*. 1962). The chromophore of GFP absorbs light of a specific range and fluoresces green light (Shimomura 1979). GFP has been transformed into cell lines to study various topics, it has many advantages in that it requires no additional substrates or cofactors for activity, just the absorption of blue light (Chalfie *et al.* 1994).

Bioluminescence resonance energy transfer (BRET) enables the monitoring of specific protein–protein interactions. This technique is based on the energy transfer that occurs when a photoprotein, such as *aequorin*, comes into close proximity with GFP or GFP-like protein. This is what occurs naturally in *Aequorea*, the greenish light that is emitted from the body of the jellyfish was not observed from the purified fractions containing the luciferin and the photoprotein involved, as blue light was observed. The study of jellyfish bioluminescence contributed to the discovery and isolation of the GFP (Shimomura *et al*. 1962). BRET can only occur when the primary light emitter and the fluorescent protein are in close proximity within (< 100 Å) (Morise *et al*. 1974). BRET has been used to monitor insulin receptor dimerization (Issad *et al.* 2002), *in vivo* DNA transcription factor binding (Germain-Desprez *et al.* 2003). Recently BRET has been used to observe the protein–protein interactions within deep tissues of mice. Mice were first transformed with both *Renilla* luciferase and fluorescent proteins. Then, the mice were injected with coelenterazine into the blood stream, and imaged for luminescence (Dragulescu-Andrasi *et al.* 2011).

Bioluminescence can also be used to study gene transcription as a reporter gene. For example, the Cytochrome P450 3A4 (CYP34A) gene activity was monitored in mice by transformation of a plasmid constructed with a reporter gene containing the P450 promoter driving the firefly luciferase coding sequence. In order to observe bioluminescence, luciferin must be injected into the animal (Zhang *et al*. 2003). The use of two bioluminescent reporter genes in the same organism has been performed to measure both oxidative and genotoxic damage in *E. coli* (Mitchell & Gu 2003). Bioluminescence imaging has also been used to study infectious diseases, such as salmonella, where salmonella transformed with the bacterial *lux* operon are injected into mice. Low yet substantial light can be detected through the animal tissue with ultra sensitive cameras, which enables monitoring of the spread of the salmonella infection (Francis *et al*. 2000). Similarly, genetically engineered bioluminescent tumours have been implanted into mice to observe the spread of the tumour non-invasively and in real-time (Yu *et al.* 2003). Similarly, transplanted cardiac stem cells can be monitored non-invasively and in real-time using bioluminescence (Wu *et al*. 2003).

Bioluminescent systems have been used in biosensors, both with immobilized enzymes on probes and with whole-cell biosensors. With immobilized enzyme probes, the luciferase protein system is coupled to another enzyme, such as the detection of lactate, where immobilized bacterial luciferase and long-chain aldehyde reductase complex are coupled to a third immobilized enzyme lactate dehydrogenase that produces NADH (Roda *et al.* 1991). Whole cell biosensors can also be engineered by inserting bioluminescence systems into various cell types such as *E. coli.* These biosensors are then exposed to the environment and varying levels of luminescence are observed depending on the target analyte concentration. This method has been used to determine the levels of arsenic, copper, cadmium, lead, chromium, mercury and various other organic compounds (Daunert *et al.* 2000). Through a signal transduction cascade, biosensors can be engineered to luminesce when exposed to specific receptor ligands (Fairey & Ramsdell 1999). There are also bio-industrial production applications, in which bioluminescent bacteria offer a non-invasive method of monitoring cell growth and density in industrial fermenters without sampling the reaction, and avoiding a potential dangerous opportunity for contamination (Trezzani *et al.* 2003).

As more research continues on bioluminescent systems, new and exciting methods will appear. There is a desire to engineer luciferases and photoproteins that do not require exotic luciferin substrates so that they can be utilized by any cell type, without exogenous augmentation of substrate.

Conclusions: the authors note on astrobiology and bioluminescence

Bioluminescence on Earth is a very common phenomena, it is almost as common as life itself, occurring in almost every major domain of life. If an environment is found on another planet that is similar to ours, including liquid water and molecular oxygen, then it is probably likely to have bioluminescence as well. This is based on the predisposition of life to bioluminesce here on Earth. It is a very common form of communication, especially in dark places and could play a role in the early evolution of organisms in these locales, and could potentially be common across many planets. Behaviours observed on Earth involving bioluminescence could be similar to those found in alien bioluminescence, such as those seen in the mesopelagic zone of oceans. Biotechnological applications of bioluminescence could aid in the detection of life and the study of it.

References

Abrahams, M.V. & Townsend, L.D. (1993). Bioluminescence in Dinoglagellates: a test of the burglar alarm hypothesis. *Ecology* **74**(1), 258–260.

Airth, R.L. & Foerster, G.E. (1962). The isolation of catalytic components required for cell-free fungal bioluminescence. *Arch. Biochm. Biophys*. **92**, 567–573.

Airth, R.L. & McElroy, W.D. (1959). Light emission from extracts of luminous fungi. *J. Bacteriol*. **77**, 249–250.

Bae, Y.M. & Hastings, J.W. (1994). Cloning, sequencing and expresssion of dinoflagellate luciferase DNA from a marine alga, *Gonyaulax polyedra*. *Biochim. Biophys. Acta* **1219**, 449–456.

https://doi.org/10.1017/S1473550412000146 Published online by Cambridge University Press

Bellisario, R., Spencer, T.E. & Cormier, M.J. (1972). Isolation and properties of luciferase, a non-heme peroxide from the bioluminescent earthworm, *Diplocardia longa*. *Biochemistry* **11**, 2256–2266.

Berkel, W.J.H., Kamerbeek, N.M. & Fraaije, M.W. (2006). Favoprotein monooxygenases, a diverse class of oxidative biocatalysts. *J. Biotechnol.* **124**, 670–689.

Bowden, B.J. (1950). Some observations on a luminescent freshwater limpet from New Zealand. *Biol. Bull.* **99**, 373–380.

Campbell, A.K. & Herring, P.J. (1990). Imidazopyrazine bioluminescence in copepods and other marine organisms. *Mar. Biol.* **104**, 219–225.

Chalfie, M., Tu, Y., Euskirchen, G. & Ward, W.W. (1994). Green fluorescent protein as a marker for gene expression. *Science* **263**, 802–805.

Conti, E., Franks, N.P. & Brick, P. (1996). Crystal structure of firefly luciferase throws light on a superfamily of adenylate-forming enzymes. *Structure* **4**(3), 287–298.

Cormier, M.J. & Strehler, B.L. (1953). The identification of KCF: requirement of long-chain aldehydes for bacterial extract luminescence. *Am. Chem. Soc.* **75**, 4864–4865.

Daunert, S., Barrett, G., Feliciano, J.S., Shetty, R.S., Shrestha, S. & Smith-Spencer, W. (2000). Genetically engineered whole-cell sensing systems: coupling biological recognition with reporter genes. *Chem. Rev.* **100**, 2705–2738.

Deheyn, D.D. & Latz, M.I. (2009). Internal and secreted bioluminescence of the marine polychaete *Odontosyllis phosphorea* (Syllidae). *Invertebr. Biol.* **128**(1), 31–45.

Desjardin, D.E., Oliveira, A.G. & Stevani, C.V. (2008). Fungi bioluminescence revisited. *Photochem. Photobiol. Sci.* **7**, 170–182.

Desjardin, D.E., Perry, B.A., Lodge, D.J., Stevani, C.V. & Nagasawa, E. (2010). Luminescent Mycena: new and noteworthy species. *Mycologia*. **102**, 459–477.

Dragulescu-Andrasi, A., Chan, C.T., De, A., Massoud, T.F. & Gambhir, S.S. (2011). Bioluminescence resonance energy transfer (BRET) imaging of protein–protein interactions within deep tissues of living subjects. *Proc. Natl. Acad. Sci. U.S.A.* **108**(29), 12060–12065.

Dunlap, J.C., Hastings, J.W. & Shimomura, O. (1980). Crossreactivity between the light-emitting systems of distantly related organisms: novel type of light-emitting compound. *Proc. Natl. Acad. Sci. U.S.A.* **77**, 1394–1397.

Eckstein, J.W., Hastings, J.W. & Ghisla, S. (1993). Mechanism of bacterial bioluminescence: 4a,5-dihydroflavin analogs as models for luciferase hydroperoxide intermediates and the effect of substituents at the 8-position of flavin on luciferase kinetics. *Biochemistry* **32**, 404–411.

Fairey, E.R. & Ramsdell, J.S. (1999). Reporter gene assays for algal-derived toxins. *Nat. Toxins* **7**, 415–421.

Francis, K.P., Joh, D., Bellinger-Kawahara, C., Hawkinson, M.J., Purchio, T.F. & Contag, P.R. (2000). Monitoring bioluminescent *Staphylococcus aureus* infections in living mice using a novel luxABCDE construct. *Infect. Immun.* **68**, 3594–3600.

Gatenby, J.B. (1959). Notes on the New Zealand Glow-worm, Bolitophila (Arachnocampa) luminosa. *Trans. R. Soc. N. Z.* **87**, 291–314.

Germain-Desprez, D., Bazinet, M., Bouvier, M. & Aubry, M. (2003). Oligomerization of transcriptional intermediary factor 1 regulators and interaction with ZNF74 nuclear matrix protein revealed by bioluminescence resonance energy transfer in living cells. *J. Biol. Chem.* **278**(25), 22367–22373.

Harvey, E.N. (1952). *Bioluminescence*. Academic Press, New York.

Issad, T., Boute, N. & Pernet, K. (2002). A homogeneous assay to monitor the acivity of the insulin receptor using bioluminescence resonance energy transfer. *Biochem. Pharmacol.* **64**, 813–817.

Kenaley, C.P. (2010). Comparative innervation of Cephalic photophores of the loosejaw dragonfishes (Teleostei: Stomiiformes: Stomiidae): evidence for parallel evolution of long-wave bioluminescence. *J. Morphol.* **271**, 418–437.

Kishi, Y., Goto, T. & Hirata, Y. (1966). Cypridina bioluminescence I: structure of *Cypridina* luciferin. *Tetrahedron Lett.* **29**, 3427–3436.

Kojima, S. *et al.* (2000). Purification and characterization of the luciferase from the freshwater snail Latia. In *Abstracts of 11th International Symposium on Bioluminescence and Chemiluminescence*, *Asilomar*, *CA*, p. 57.

Liu, Y., Cotton, J.A., Shen, B., Han, X., Rossiter, S.J. & Zhang, S. (2010). Convergent sequence evolution between echolocating bats and dolphins. *Curr. Biol.* **20**, R53–54.

Meighen, E.A. (1991). Molecular biology of bacterial bioluminescence. *Microbiol. Mol. Biol. Rev.* **55**(1), 123–141.

Meighen, E.A. (1994). Genetics of bacterial bioluminescence. *Annu. Rev. Genet.* **28**, 117–139.

Mitchell, R.J. & Gu, M.B. (2003). An *Escherichia coli* biosensor capable of detecting both genotoxic and oxidative damage. *Appl. Microbiol. Biotechnol.* **64**, 46–52.

Morin, J.G. (1983). Coastal bioluminescence: patterns and functions. *Bull. Mar. Sci.* **33**, 787–817.

Morise, H., Shimomura, O., Johnson, F. H., & Winant, J. (1974). Intermolecular energy transfer in the bioluminescent system of Aequorea. *Biochemistry* **13**, 2656–2662.

Nakajima, Y., Kobayashi, K., Yamagishi, K., Entomoto, T. & Ohmiya, Y. (2004). cDNA cloning and characterization of a secreted luciferase from the luminous Japanese ostracod, *Cypridina noctiluca*. *Biosci. Biotechnol. Biochem.* **68**, 565–570.

Nakamura, H., Musicki, B., Kishi, Y. & Shimomura, O. (1988). Structure of the light emitter in krill *Euphausia pacifica*. *J. Am. Chem. Soc.* **110**, 2683–2685.

Nakamura, H., Kishi, Y., Shimomura, O., Morse, D. & Hastings, J.W. (1989). Structure of dinoflagellate luciferin and its enzymatic and nonenzymatic air-oxidation products. *J. Am. Chem. Soc.* **111**, 7607–7611.

Ohtsuka, H., Rudie, N.G. & Wampler, J.E. (1976). Structural identification and synthesis of luciferin from the bioluminescent earthworm, *Diplocardia longa*. *Biochemistry* **15**, 1001–1004.

Oliveira, A.G. & Stevani, C.V. (2009). The enzymatic nature of fungal bioluminescence. *Photochem. Photobiol. Sci.* **8**, 1416–1421.

Oliveira, A.G., Desjardin, D.E., Perry, B.A. & Stevani, C.V. (2012). Evidence that a single bioluminescent system is shared by all known bioluminescent fungal lineages. *Photochem. Photobiol. Sci.* DOI: 10.1039/C2PP25032B.

Ow, D.W., Wood, K.V., DeLuca, M., De Wet, J.R., Helinski, D.R. & Howell, S.H. (1986). Transient and stable expression of the firefly luciferase gene in plant cells and transgenic plants. *Science* **234**, 856–859.

Roda, A., Girotti, S., Grigolo, B., Ghini, S., Carrea, G., Bovara, R., Zini, I. & Grimaldi, R. (1991). Microdialysis and luminescent probe: analytical and clinical aspects. *Biosens. Bioelectron.* **6**, 21–29.

Roda, A., Pasini, P., Mirasoli, M., Michelini, E. & Guardigli, M. (2004). Biotechnological applications of bioluminescence and chemiluminescence. *Trends Biotechnol.* **22**, 295–303.

Rudie, N.G., Mulkerrin, M.G. & Wampler, J.E. (1981). Earthworm bioluminescence: characterization of high specific activity *Diplocardia longa* luciferase and the reaction it catalyzes. *Biochemistry* **20**, 344–350.

Schultz, L.W., Lie, L., Cegielski, M. & Hastings, J.W. (2005). Crystal structure of a pH-regulated luciferase catalyzing the bioluminescent oxidation of an open tetrapyrrole. *Proc. Natl. Acad. Sci. U.S.A.* **102**, 1378–1383.

Seliger, H.H. & McElroy, W.D. (1964). The colors of firefly bioluminescence: enzyme configuration and species specificity. *Proc. Natl. Acad. Sci. U.S.A.* **52**, 75–81.

Shimomura, O. (1979). Structure of the chromophore of Aequorea green fluorescent protein. *FEBS Lett.* **104**, 220–222.

Shimomura, O. (1995). The roles of the two highly unstable components F and P involved in the bioluminescence of euphausiid shrimps. *J. Biolumin. Chemilumin.* **10**, 91–101.

Shimomura, O. (2006). *Bioluminescence: Chemical Principles and Methods*. World Scientific, Singapore.

Shimomura, O. & Johnson, F.H. (1968a). The structure of Latia luciferin. *Biochemistry* **7**, 1734–1738.

Shimomura, O. & Johnson, F.H. (1968b). Purification and properties of the luciferase and of a protein cofactor in the bioluminescence system of Latia neritoides. *Biochemistry* **7**, 2574–2580.

Shimomura, O. & Johnson, F.H. (1970). Mechanisms in the quantum yield of Cypridina bioluminescence. *Photochem, Photobiol.* **12**, 291–295.

Shimomura, O. & Johnson, F.H. (1971). Mechanism of the luminescent oxidation of Cypridina luciferin. *Biochem. Biophys. Res. Commun*. **44**, 340–346.

Shimomura, O., Johnson, F.H. & Saiga, Y. (1962). Extraction, purification and properties of aequorin, a bioluminescent protein from the luminous hydromedusan, aequorea. *J. Cell. Comp. Physiol*. **59**, 223–239.

Shimomura, O., Goto, T. & Johnson, F.H. (1977). Source of oxygen in the CO_2 produced in the bioluminescent oxidation of firefly luciferin. *Proc. Natl. Acad. Sci. U.S.A.* **74**, 2799–2802.

Siewers, V., Smedsgaard, J. & Tudzynski, P. (2004). The P450 monooxygenase BcABA1 is essential for abscisic acid biosynthesis in *Botrytis cinerea*. *Appl Environ. Microbiol*. **70**(7), 3868–3876.

Sivinski, J. (1981). Arthropods attracted to luminous fungi. *Psyche* **88**, 383–390.

Sivinski, J. (1998). Phototrophism, bioluminescence, and the diptera. *Florida Entomol*. **81**(3), 282–292.

Stevani, C.V. & Baader, W.J. (1999). O sistema quimiluminescente peróxi-oxalato. *Quim. Nova* **22**, 715–723.

Stojanovic, M.N. & Kishi, Y. (1994). Dinoflagellate bioluminescence: the chemical behavior of the chromophore towards oxidants. *Tetrahedron Lett*. **35**, 9347–9350.

Szent-Gyorgyi, C., Ballou, B.T., Dagnal, E. & Bryan, B. (1999). Cloning and characterization of new bioluminescent proteins. In *Proceedings of SPIE-The International Society for Optical Engineering (Biomedical Imaging: Reporters, Dyes, and Instrumentation*), p. 4.

Sutton, T.T. (2005). Trophic ecology of the deep-sea fish *Malacosteus niger* (Pisces: Stomiidae): an enigmatic feeding ecology to facilitate a unique visual system. *Deep Sea Res*. **52**, 2065–2076.

Thompson, E.M., Nagata, S. & Tsuji, F.I. (1989). Cloning and expression of cDNA for the luciferase from the marine ostracod Vargulahilgendorfii. *Proc. Natl. Acad. Sci. U.S.A.* **86**, 6567–6571.

Trezzani, I., Nadir, M., Dorel, C., Lejeune, P., Bellalou, J., Lieto, J., Hammouri, H., Longin, R. & Dhurjati, P. (2003). Monitoring of recombinant protein production using bioluminescence in a semiautomated fermentation process. *Biotechnol. Prog*. **19**, 1377–1382.

Tu, S.C. (2007). Activity coupling and complex formation between bacterial luciferase and flavin reductases. *Photochem. Photobiol. Sci*. **7**, 183–188.

Tudzynski, B. (2005). Gibberellin biosynthesis in fungi: genes, enzymes, evolution, and impact on biotechnology. *Appl. Microbiol. Biotechnol*. **66** (6), 597–611.

Villa, R. & Willetts, A. (1997). Oxidations by microbial NADH plus FMN-dependent luciferases from Photobacterium phosphoreum and Vibrio fischeri. *J. Mol. Catal*. **2**, 193–197.

Viviani, V.R. (2002). The origin, diversity, and structure function relationships of insect luciferases. *Cell. Mol. Life Sci.*. **59**, 1833–1850.

Viviani, V.R., Silva, A.C.R., Perez, G.L.O., Santelli, R.V., Bechara, E.J.H. & Reinach, F.C. (1999). Cloning and molecular characterization of the cDNA for the Brazilian larval ciick-beetle *Pyrearinus termitilluminans* luciferase. *Photochem. Photobiol*. **70**, 254–260.

Watanabe, H. & Hastings, J.W. (1982). Specificities and properties of three reducted pyridine nucleotide-flavin mononucleotide reductases coupling to bacterial luciferase. *Mol. Cell Biochem*. **44**, 181–187.

White, E.H., McCapra, F., Field, G. & McElroy, W.D. (1961). The structure and synthesis of firefly luciferin. *J. Am. Chem. Soc*. **83**, 2402–2403.

Widder, E.A. (2001). Bioluminescence and the Pelagic visual environment. *Mar. Fresh. Behav. Physiol*. **35**, 1–26.

Wilson, T. & Hastings, J.W. (1998). Bioluminescence. *Annu. Rev. Dev. Biol*. **14**, 197–230.

Wood, K.V., De Wet, J.R., Dewji, N. & DeLuca, M. (1984). Synthesis of active firefly luciferase by *in vitro* translation of RNA obtained from adult lanterns. *Biochem. Biophys. Res. Commun*. **124**, 592–596.

Wood, K.V., Lam, Y.A., Seliger, H.H. & McElroy, W.D. (1989). Complementary DNA coding click beetle luciferase can elicit bioluminescence of different colors. *Science* **244**, 700–702.

Wu, J.C., Chen, I.Y., Sundaresan, G., Min, J.J., De, A., Qiao, J.H., Fishbein, M.C. & Gambhir, S.S. (2003). Molecular imaging of cardiac cell transplantation in living animals using optical bioluminescence and positron emission tomography. *Circulation* **108**, 1302–1305.

Yu, Y.A., Timiryasova, T., Zhang, Q., Beltz, R. & Szalay, A.A. (2003). Optical imaging: bacteria, viruses, and mammalian cells encoding light-emitting proteins reveal the locations of primary tumors and metastases in animals. *Anal. Bioanal. Chem*. **377**, 964–972.

Zenno, S. & Saigo, K. (1994). Identification of the genes encoding NAD(P)H flavin oxidoreductases that are similar in sequence to *Escherichia coli* Fre in four species of luminous bacteria Photohabdus luminescens, *Vibrio fischeri, vibrio harveyi* and *vibrio orientalis*. *J. Bacteriol* **176**, 3544–3551.

Zhang, W., Purchio, A.F., Chen, K., Wu, J., Lu, L., Coffee, R., Contag, P.R. & West, D.B. (2003). A transgenic mouse model with a luciferase reporter for studying *in vivo* transcriptional regulation of the human CYP3A4 gene. *Drug Metab. Dispos*. **31**, 1054–1064.