New Perspectives Related to the Bioluminescent System in Dinoflagellates

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Version received: 24 March 2020

The mechanisms underlying the bioluminescent phenomenon have been well characterized in dinoflagellates; however, there are still some aspects that remain an enigma. Such is the case of the presence and diversity of the luciferin-binding protein (LBP), as well as the synthesis process of luciferin. We carry out a review of the literature in relation to the molecular players responsible for bioluminescence in dinoflagellates, with particular interest in *P. lunula*.

Dinoflagellates are the most important eukaryotic protists that produce light [1][2]. This singularity has inspired not only literature and art, but also an intensive scientific dissection [3][4][5]. *Pyrocystis* has been a main model genus for a long time in the study of bioluminescence in dinoflagellates [6][7][8][9][10]; as well as in the development of some biotechnological applications associated with its bioluminescence capacity [11][12][13].

All dinoflagellates belong to the Dinophyceae group and have been unchallengeably placed using extensive molecular phylogenetic data within the Alveolata group, being closely related to the Apicomplexa group, which includes many parasitic species [14]. *Pyrocystis* (Dinophyceae) spends a large part of its life as a non-mobile cell on a shell covered with cellulose [15][16]. *Pyrocystis* includes a small number of marine species that have a cosmopolitan distribution [17]. The life cycles of *P. lunula*, as in other species of this genus, it is characterized by a normal asexual reproduction linked to simple alternations of coccoid cells and morphologically different transitory reproductive stages. There are different reproductive bodies depending of the species. In the case of *P. lunula*, the reproductive bodies are athecate-uniflagellate planospores. In *P. noctiluca* and *P. fusiforrnis* are athecate aplanospores. For *P. lanceolate* are athecate-biflagellate, and in *P. acuta* are thecate-biflagellate [18]. Furthermore, evidence of sexual reproduction has been reported in *P. lunula* [19]. The *P. lunula* lifestyle is also characterized by the execution of vertical migrations in relation to the circadian rhythm [20].

The bioluminescent light is generated by a chemical reaction. Although the process is not the same in all the bioluminescent organisms, most of them share the same base reaction; where the LCF enzyme reacts with the luciferin (substrate) in presence of oxygen and produces an oxyluciferin that emits a photon while it decays from a high to a low energy state. There are exceptions to this base reaction, for example, in some luminous earthworms [5] and acorn worms [21], the bioluminescent event is triggered by H_2O_2 and not for O_2. Besides, the electronic structures, absorption and fluorescence spectra of luciferin, its six analogues and its oxidized form, "oxyluciferin" showed clear evidence of the lack of fluorescence in *Latia neritoides* [22].

The cellular mechanisms and the genes involved with bioluminescence in dinoflagellates are well characterized. The bioluminescence system in these organisms is unique, from a molecular and cellular point of view. The production of light takes place in specialized organelles, the scintillons, which contain the LCF enzyme, the substrate luciferin, and in most cases a LBP [23][24][25][26]. The light emission is based on LCF-catalyzed oxidation of the luciferin, generally protected from oxidation by LBP that binds the luciferin at physiological pH (Figure 1). Furthermore, molecular studies have demonstrated a high variation in the sequences of LBP, showing a highly diverse gene family including several non-identical copies arranged in tandem within the genome [27][28], like in *Lingulodinium polyedra* [27][29][30], *Noctiluca scintillans* [31], *Alexandrium spp* [32][33][34], and *Pyrocystis lunula* [35]. The LBP has also been found in the genera *Gonyaulax*, *Ceratocorys*, *Protoceratium* [36][37].

Until recently, it was thought that the genus *Pyrocystis* was among the few ones lacking the presence of the LBP [23][25][37] such as in genera *Ceratium*, *Fragilidium*, and *Protoperidinium* [37]; however, this fact was refuted by the recent detection and characterization of LBP in *P. lunula* [38]. Emerging information shows substantial evidence that LBP is an integral component of the standard molecular bioluminescence system in dinoflagellates [38][37][39].

Another important fact that today remains an enigma refers to what is the exact mechanism underlying the luciferin synthesis process. In *P. lunula*, in contrast to other bioluminescent dinoflagellates, the levels of LCF and luciferin are constant throughout the daily cycle [25]. Therefore, the rhythm is related to changes in their intracellular localization, instead of daily *de novo* synthesis and destruction of all the components [16][40]. According to available evidence, it has been proposed that luciferin can be synthesized through different ways, and is thought to be universal in dinoflagellates, because luciferin from any dinoflagellate bioluminescent species can be used as subtract to produce light [41]. It was suggested that luciferin is a photo-oxidation breakdown product of chlorophyll a [42]; however, this would not be true in all cases since *L. polyedra* only contains luciferin during the night period when photo-oxidation is not possible; and *Protoperidinium crassipes* can preserve even one year its bioluminescence in the absence of food with chlorophyll or luciferin [43] and, therefore, it can be suggested that it contains luciferin originated from a different precursor. It is likely that more than one mechanism is responsible for luciferin production [44]. In fact, a study with amino acid tracers has confirmed the intracellular production of luciferin , and in this regard, Fresneau and Arrio [44] argue that bioluminescence in dinoflagellates is ruled by the reduction state of the luciferin precursor. Regarding the controversies in relation to these issues, and due to the ecological importance of Dinophyceae in marine environments and to the bioluminescence as a strategy for competition and/or survival, we have made a comprehensive literature review and metanalysis that explores the current available knowledge in relation to the function of bioluminescence in dinoflagellates and the description of the key players involved in the production of bioluminescence. It also gives some new perspectives regarding the phylogenetic diversity/conservation of protein sequence, structure and evolutionary pattern of these key players.

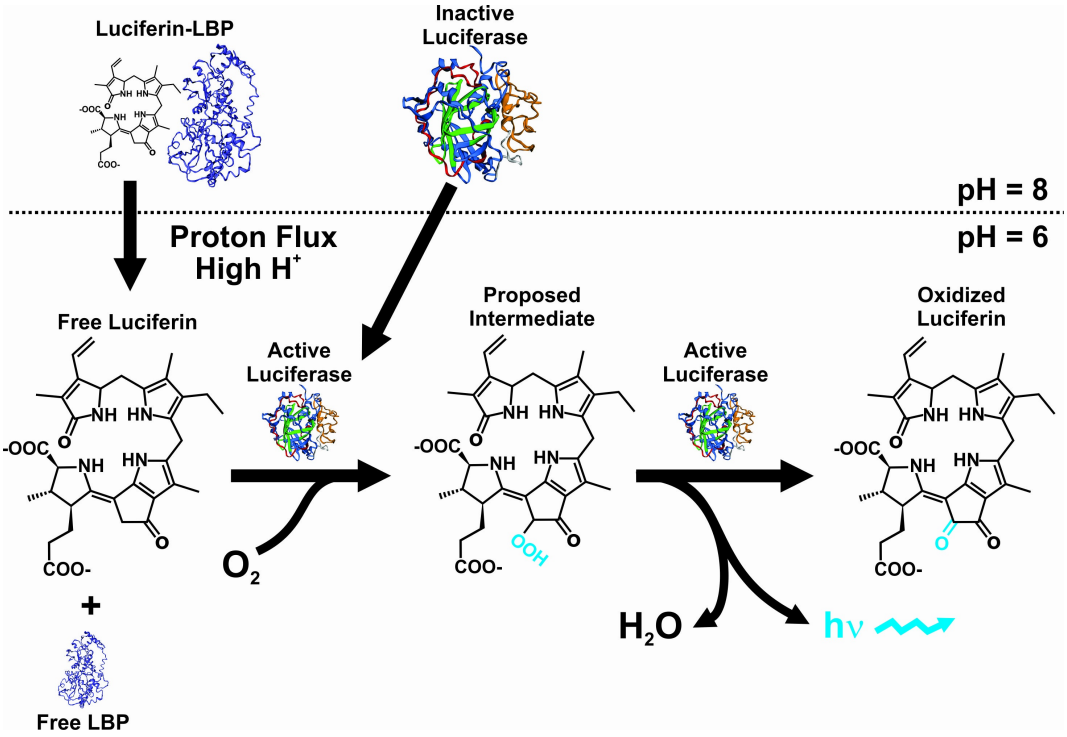

Figure 1. Bioluminescence model in dinoflagellates, showing the efect of pH on both LBP and LCF. Modified from the proposed model by Rüdiger Hardeland (http://tolweb.org/notes/?note_id=5621) and work published by Morse et al [30].

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Keywords

luciferase; luciferin-binding protein; luciferin; P630; blue compound; glutathione S-transferase