Short communication

Aequorin-expressing yeast emits light under electric control

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In this study, we show the use of direct external electrical stimulation of a jellyfish luminescent calcium-activated protein, aequorin, expressed in a transgenic yeast strain. Yeast cultures were electrically stimulated through two electrodes coupled to a standard power generator. Even low (1.5 V) electric pulses triggered a rapid light peak and serial light pulses were obtained after electric pulses were applied periodically, suggesting that the system is re-enacted after a short refraction time. These results open up a new scenario, in the very interphase between synthetic biology and cybernetics, in which complex cellular behavior might be subjected to electrical control.

Keywords: Aequorin, Bioluminescence, Yeast electro-stimulation, Synthetic biology, Bioelectronics

The interface between biology and electronics is a potent yet unexplored field of synthetic biology. In order to bridge the gap between these two disciplines, a greater understanding should be sought of the interaction between electronic devices and biological components. Electrical impulses are a native language of electronics and are thus well suited for such interactions. Unfortunately, cells which are naturally equipped with the ability to respond to electrical signals chemically – neurons and muscle cells (Requena et al., 1991; Watanabe and Endoh, 1998) – are difficult to culture, (Takahashi et al., 1999; Roda et al., 2004) – to forecast the potential of external electric pulses as a control device of light emission in genetically engineered S. cerevisiae strains. Aequorin is composed of two distinct units (Shimomura et al., 1962): apoequorin, with an approximate molecular weight of 22 kDa and the prosthetic group, coelenterazine, a luciferin. The aequorin–coelenterazine complex is sensitive to Ca2+ because addition of calcium to the complex triggers fast oxidation of coelenterazine to coelenteramide, which results in the emission of blue light when coelenteramide relaxes to the ground state (Shimomura et al., 1963).

A recombinant yeast strain expressing aequorin was grown in selective Leu-SD (synthetic minimal medium lacking Leucine) and incubated with coelenterazine as previously described (Viladevall et al., 2004). Then it was subjected to a controlled depolarization of the cell membrane with KOH, resulting in light emission as recorded by TD-20e luminometer (Turner BioSystems) (Fig. 1). Inspired by the adaptation of the Hodgkin–Huxley model, traditionally used to describe the dynamics of electrical pulses in neurons, we hypothesized that an effective transmembrane potential of a few volts would produce a similar response in yeast (Catterall, 2000; Cui and Kaandorp, 2006). Indeed, the application of low-voltage (1.5–16 V) electric pulses for 1–5 s triggered a very similar behavior in terms of light emission, as deduced by the sharp peaks
observed shortly after electro-stimulation (Fig. 1). As expected, electrically induced bioluminescence was calcium-dependent as confirmed by a series of experiments in which electro-stimulation was performed with yeast suspensions containing calcium chelating agents such as EDTA, which retrieved no light emission (Fig. 2). Thus, free calcium ions must be present in the extracellular medium in order for yeast cells to glow. Further control reactions lacking aequorin, coelenterazine, or yeast suspensions, were also electrically stimulated and again no light production was retrieved. Taken together, these results discard the possibility that light production is an artifact, and confirm that light emission requires the integrity of the whole calcium-based system.

Under our experimental conditions, light emission was reproducible after a short refraction time (Fig. 2), indicating that the mechanism underlying calcium entry is triggered by the electric pulse, deactivated when no electricity is applied, and ready for reenactment by an ulterior pulse. The intensity of light peaks was, at least under the tested conditions (from 2 to 9 V), pulse-dependent (Fig. 3).

From our results, it is tempting to hypothesize that external electric pulses induce voltage-dependent calcium channel opening and

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**Fig. 1.** Alkali-triggered stimulation (dashed line) and electro-stimulation (continuous line) of yeast strains showing very similar behavior. Alkali-based stimulation was performed at \( t = 5 \) s by adding 30 \( \mu l \) of KOH 100 mM to 170 \( \mu l \) of an aequorin-expressing yeast suspension. Electro-stimulation was carried out at \( t = 5 \) s by supplying 6 V during 5 s to another aequorin-expressing yeast suspension.

**Fig. 2.** Response of genetically engineered yeasts expressing aequorin to electrical stimulation. Cell suspensions grown as previously described (Viladevall et al., 2004) were incubated in Leu-SD medium with coelenterazine for 5 h and subjected to a 6 V electric pulse of 5 s of duration every minute. Arbitrary units are shown. Control reactions lacking coelenterazine (\( Aeq^+, \ coe^- \)), yeast (\( SD, \ coe^+ \)), and lacking both coelenterazine and yeast (\( SD, \ coe^- \)), along with a chelating (EDTA)-containing reaction (\( Aeq^+, \ coe^+, \ EDTA^+ \)) were used. Additionally, wild-type yeast strains without aequorin, with (\( Aeq^-, \ coe^- \)) and without (\( Aeq^-, \ coe^- \)) coelenterazine were assayed.

**Fig. 3.** Pulse-response of aequorin-expressing yeast suspensions prepared as described in the main text and subjected to electrical pulses of 2, 6, and 9 V, for 5 s. Luminescence given in arbitrary units.
subsequent massive calcium entry, which results in a light peak as a consequence of calcium binding to the aequorin–coelenterazine complex. However, when calcium channel mutant strains mid1 and cch1 (Viladevall et al., 2004) were subjected to electro-stimulation, significant light production was also observed (data not shown). This is an indication that the externally induced membrane depolarization does not mimic alkali-based depolarization. A different mechanism might thus lie behind light production under external electrical stimulation. One possibility is that remaining calcium channels (and maybe alternative calcium-transporting proteins) might facilitate non-physiological calcium ions entry under artificial depolarization. However, it cannot be ruled out that, similarly to the mechanism supposed to be responsible of electroporation for transforming competent cells with exogenous DNA, electro-stimulation might also trigger transient channel-independent pores to open through the cell membrane. In any case, the fact that bioluminescence can be observed without loss of intensity over repeated applications of the voltage (Fig. 2) suggests electrical stimulation is not harmful to the cells. The quick exponential fall in the bioluminescence signal suggests an active mechanism of calcium sequestration, probably by cellular organelles such as vacuoles or endoplasmic reticulum, ruling out cell lysis as a reason for increased luminescence of aequorin.

The present work is the first step towards electrically controlling bioluminescence, opening two novel research directions: first, the implementation of novel bio-electronic lighting devices, including displays with living cells as pixels and second, the possibility of electrically controlling the behavior of GMOs. Given our results, it can be concluded that it is theoretically possible to implement a biological light display with light emission (for a simulation of a simple display based on real light emitting yeasts, see supplementary material Video 1 online). Regarding the electrical control of cell physiology, further studies are needed in order to forecast the potential for controlling gene expression, enzymatic activity or any other desired behavior in GMOs by triggering the massive and re-enactable entry of ions by artificial electrical stimulation.

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**Appendix A. Supplementary data**

Supplementary data associated with this article can be found, in the online version, at doi:10.1016/j.jbiotec.2011.01.005

**References**


