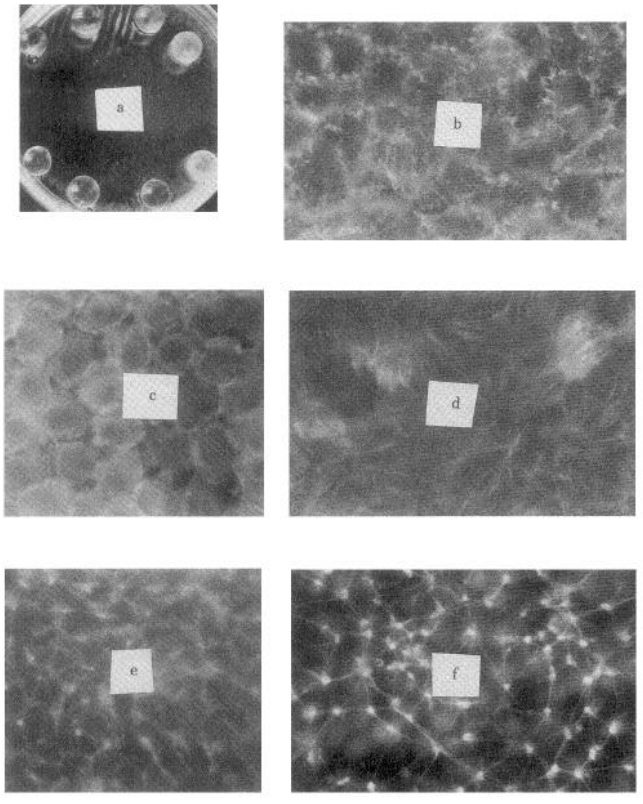

Figure 1. *Morphology and microanatomy of dogfish lenses exposed to UV-A radiation with or without modifiers. a) Opacities in whole lenses after 15 h of incubation: Lower row (left to right) dark control; with 0.1 mM HT; with 1 mm HT; with 10 mM HT; upper row (left to right) UV-exposed; with 0.1 mM HT; with 1 mM HT and with 10 mM HT. (b–f) Rhodamine-phalloidin stained dogfish lens epithelial cells in whole mount (magnification 5250×) after 15 h incubation. b) Dark control; c) UV-exposed; d) UV-exposed plus α-tocopherol added at 25 μM; e) dark control plus 10 mM OH-TEMPO; f) UV-exposed plus 10 mM OH-TEMPO added.*

depleted in the UV-exposed lenses by about 30–40% compared with dark controls. Thus, UV-A exposure lowered the ATP level concomitant with opacity formation.

UV-A-exposed lens epithelial cells contained few if any actin filaments, whereas in the dark controls filaments were prominent (see Fig. 1b, c). The degree of loss of these actin filaments due to UV-exposure was lessened when vitamin E was present in the medium (see Fig. 1d). However, OH-TEMPO caused a greater opacity and a greater degree of alteration of the actin filaments (Fig. 1e, f). When OH-TEMPO was added at 10 m*M*, a totally aberrant pattern of actin distribution was observed. The fluorescence due to phalloidin-rhodamine binding to actin was concentrated in intense spots near the lens cell membranes and interconnecting rays of filaments, forming star-like figures. Thus, opacification, ATP depletion, and F-actin breakdown all result from UV-A irradiation.

Purified rabbit muscle F-actin was also degraded by 5–20% into monomeric fragments due to UV-irradiation. HPLC data indicated that α-tocopherol (at 25 μ*M*) neither protected nor enhanced UV-induced F-actin degradation.

We conclude that a photooxidative process degrades filamentous actin, and that α-tocopherol partially protects it in cells, whereas OH-TEMPO (the spin label trap) perhaps by acting as a photosensitizer, enhances this degradation. As α-tocopherol is known to be a free radical scavenger, its protective action may be related to quenching the UV-induced production of free radicals. The action of OH-TEMPO (a free radical) could be the result of a free radical attack on the actin polymer, leading to partial destabilization. The role of ATP diminution in this process is being investigated.

Supported by NIH (EY 00698 and EY 00459) and RPB, Inc.

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Towards the Development of a Technique for Introducing Aequorin into Amoebae of *Dictyostelium discoideum* Via Electroporation

Dawn M. Cishek (Marine Biological Laboratory), Richard H. Sanger, and Lionel F. Jaffe

Aequorin is a 22,000 molecular weight luminescent photoprotein that emits light at 470 nm when mixed with trace amounts of free calcium. It has been used extensively to image free calcium patterns, pulses, and waves as they occur in development (1). Ordinarily, aequorin is introduced into the cytosol of a single, large cell by microinjection. We are developing a technique by which aequorin can be mass-introduced to a great number of small animal cells at once, via electroporation. Electroporation is a relatively new technique in which a suspension of cells is subjected to one or a series of high voltage pulses. (Conventionally, 3–5 pulses of 2–5 kV/cm for 0.1–200 ms) The delivered pulse results in an electric field across each cell. When this field exceeds the dielectric strength of the cell membrane, the membrane breaks down in localized areas, forming "pores." If the field strength and pulse length are not excessive, then the pores will reseal after a brief time, leaving the cell intact and alive. If excessive, however, the cell membrane can be irreversibly damaged and the cells will lyse. While the pores are open, small molecules from the external medium can diffuse into the cytosol. Unfortunately, aequorin is not small enough to do this. Therefore, the conventional electroporation parameters must be modified. Instead of delivering a series of medium voltage, medium length pulses, we are going to electroporate in a two step process. First we will deliver a very high, very fast pulse. Then

second, we will deliver a very long, very low voltage pulse. The theory is that the first will open pores and the second will not only keep the pores open, but also electro-osmotically drive the aequorin into the cytosol.

As a starting point, we repeated the work of Van Haastert *et al.* (1989), in which a 180 m.w. sugar was electroporated into amoebae of *D. discoideum*. We initially tried using a commercial device, but found that it could not deliver brief enough pulses for our purposes (control of pulse length is in the ms range.) The commercial equipment is intended to deliver long pulses through media several orders of magnitude less conductive than ours, therefore it is built with a very high capacitance and internal impedance. Because we were unable to modify the commercial device, we built our own. It is similar to the commercial one, but ours is able to deliver much faster pulses (*i.e.*, in the μs range as opposed to the ms range) at high voltages. (Fig. 1)

Once the equipment was built, we could begin to search for the electroporation conditions that would result in the greatest percentage of living, electroporated cells. Because aequorin is expensive and somewhat difficult to handle, we decided to begin our search using inexpensive fluorescent dyes, namely lucifer yellow CH (m.w. 487) and FITC-dextrans (m.w. 4400 and 10,000.)

Amoebae of *D. discoideum* are prepared for electroporation according to Van Haastert *et al.* (2). Cell suspensions, electroporation media, and the electroporation chamber are cooled to 0–4°C prior to electroporating. The cell suspension (0.75 ml) is mixed with the dye (0.25 ml) immediately prior to being placed in the electroporation chamber. The "chamber" is created by a 1.0 cm gap between two cylindrical aluminum electrodes, which are encased in plexiglass. The capacitors are charged to the desired voltage and the pulse is delivered through the cell suspension. The geometry of the chamber assures that a uniform field is delivered. After electroporation, the cell suspension is removed from the chamber and mixed with "healing medium" (2) and placed on ice for 10 min. Cells are washed by centrifugation

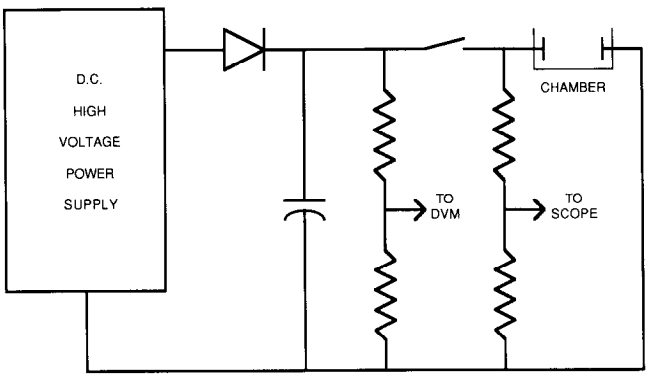

Figure 1. *Schematic of the electroporation device.*

three times in healing medium. The cells are examined for viability and uptake of dye, using both brightfield and fluorescence microscopy. Cell viability and dye uptake are the two criteria for judging the usefulness of each electroporation setting.

Using lucifer yellow, we repeated the results of Van Haastert. Under the reported optimal conditions of two 7 kV pulses for 210 μs, we attained populations in which all living cells are stained, and the number of dead cells is not statistically attributable to electroporation.

Using FITC-dextran 4400, we attained populations in which 25% of the living cells are stained, and the number of dead cells is not statistically attributable to electroporation.

The next step in this research will be to use the larger dye, FITC-dextran 10,000. Once we have attained a high electroporation efficiency with this, we will begin using aequorin.

This work was supported by NSF and NIH grants to LFJ.

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A Region of Steady High Calcium at the Vegetal Pole of Medaka Eggs

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Over a decade ago, it was conclusively demonstrated that a remarkable explosive wave of free cytosolic calcium travels from the animal pole to the vegetal pole following fertilization of the egg of the medaka fish (1). Since then it has been shown that such waves traverse a wide variety of, and perhaps all, deuterosome eggs, and may well be the explosive key that blasts the lock off the arrested development of unfertilized eggs. Such waves are propagated by calcium-induced calcium release and traverse a wide variety of eggs at a remarkably similar velocity of around 10 μm/s (2). In spite of the excitement at the confirmation of such a prominent and spectacular phenomenon, it was noted and recorded that the calcium wave lingered for some time at the vegetal pole of medaka. The significance of a domain of high calcium at the vegetal pole in early development, if indeed it was real, was subject to considerable speculation. However, such a phenomenon might be explained by two considerations that bear on the structure and early embryogenesis of the medaka egg. This large (1200 μm diameter) clear egg consists of a central membrane bound yolk mass with a thin (~40 μm thick) peripheral cytoplasm. Thus, the increased signal from the vegetal pole might be explained by the simple fact that there may be more cytoplasm at the vegetal pole or there might be an asymmetric distribution of the calcium recording molecule at this pole, perhaps explained by differential binding to some vegetal cytoskeletal element.

We have continued to use, as in the first report of calcium waves, the chemiluminescent photoprotein aequorin, as our method of choice for recording cytosolic free calcium. This