associated with NEB in fertilized cleaving eggs. It is also the first report that such pulses are absent during subsequent NEB's, do not require external calcium, and may be periodically repeated in dispermic eggs. The further use of ultrasensitive aequorins should make it possible to better characterize NEB calcium pulses and better study their function. In particular, it should now be possible to test the prediction that such pulses initially spread through the egg in the form of fast calcium waves (15).

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Calcium Buffer Injections Block Ooplasmic Segregation in *Oryzias latipes* (Medaka) Eggs

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We used the calcium buffer, 5,5'-dibromo-BAPTA, to investigate the possible role of Ca^{2+} in ooplasmic segregation in the medaka fish egg. The unfertilized medaka egg consists of two compartments: a large (~1 mm in diameter) central yolk vacuole and a thin (~30 μm thick) peripheral layer of ooplasm; a unit membrane (the yolk membrane) separates the two compartments. Following activation of the egg, the bulk of the ooplasm and its inclusions move toward the animal pole and form a blastodisc there, while oil droplets and other, smaller inclusions move toward the vegetal pole (1). At 25°C, segregation is complete within about 70 min.

Throughout the period of segregation, zones of elevated cytosolic [Ca^{2+}] are present at both the animal and vegetal poles of the medaka egg (2). To determine whether these zones are required for ooplasmic segregation, we injected dibromo-BAPTA (final concentration = 0.5–7.0 m*M*) into the ooplasm near the vegetal pole of the egg or near its equator (midpoint along the animal-vegetal axis) within 10 min after fertilization, using methods described previously (2). Our idea was to use this relatively weak calcium buffer (K_D = 1.5 μM) as a shuttle buffer to suppress or reverse the formation of needed high calcium zones in the micromolar range (3). Eggs were co-injected with sufficient Ca^{2+} to give free [Ca^{2+}] in the injectate of 0.14 μM, 0.2 μM, or 0.3 μM and thus in the range of natural resting levels (4). Injections of such buffer/Ca^{2+} mixtures, set at the resting level of [Ca^{2+}], cannot act by shifting [Ca^{2+}] away from the resting level (3). To estimate the time required for the buffer to diffuse around the egg from the site of injection, we monitored the spread of fluorescein injected near the equator of the egg. We found that it reached the poles of the egg within 10 min of injection and the antipode of the injection site within 20 min.

Low concentrations of the buffer (≤2.0 m*M*) had little or no effect on the eggs, while higher concentrations of the buffer (≥2.6 m*M*) inhibited ooplasmic segregation and blocked cleavage at all three levels of [Ca^{2+}] (Table I). The fact that the results were independent of pCa in this range confirms that the injectate was acting as a shuttle buffer. The slightly higher concentration of dibromo-BAPTA required to inhibit segregation and cleavage in the medaka egg *vs.* fucoid egg development may be due to a slightly higher calcium flux in the medaka egg.

The immediate reaction of the eggs to the injectate was an apparent expansion of the yolk membrane near the injection site, which caused the yolk vacuole to bulge into the ooplasm locally. At lower concentrations of buffer (<4 m*M*), this bulge usually subsided within 10 min. Sometimes, however, the yolk membrane over the bulge lysed; this was the predominant outcome at ≥4.0 m*M* buffer. This effect on the yolk membrane may have been caused by the initially high concentration of buffer near the injection site. Another consistent local response to the injection was the movement of nearby oil droplets away from the injection site and toward the top of the egg; this movement was over within 15 min.

Except for this early movement of oil droplets, segregation of ooplasm and oil droplets was strongly inhibited by ≥2.6 m*M* dibromo-BAPTA. The blastodisc formed slowly, and its final size was smaller than in control eggs; this effect was more pronounced in eggs in which we injected buffer near the equator. The effect of buffer injection on oil droplet movement also varied with the site of injection. When buffer was injected near the equator, oil droplet movement was strongly inhibited all along the animal-vegetal axis, but when it was injected near the vegetal pole, oil droplets moved away from both poles and formed a

Table I

Inhibition of ooplasmic segregation in the medaka egg by the injection of 5,5'-dibromo-BAPTA calcium buffer

[Free Ca^{2+}] μM	[Dibromo-BAPTA] mM*	N	Response of embryos: Lysed	Segregated normally	Cleaved	Formed embryonic axis
0.14	0.5–2.0	6	0	6	6	6
0.14	2.6–3.6	10	1	0	0	0
0.14	4.0–7.0	9	4	0	0	0
0.20	0.5–2.0	6	0	6	6	6
0.20	2.6–3.6	24	7	0	0	0
0.20	4.0–7.0	23	20	0	0	0
0.30	0.5–2.0	5	0	5	5	5
0.30	2.6–3.6	20	7	0	0	0
0.30	4.0–7.0	3	3	0	0	0

* Estimated final cytosolic concentration. See text.

ring just above the equator of the egg. Buffer concentrations ≥2.6 mM buffer also blocked cleavage. Control eggs and eggs in which we injected the buffer into the yolk vacuole developed normally.

In a previous study (2), 2.7 mM dibromo-BAPTA was shown to facilitate the diffusion of Ca^{2+} away from the poles of the medaka egg and thus substantially dissipate calcium gradients near them. The results of the present study suggest that these zones are necessary for the normal segregation of ooplasm and its inclusions in this egg.

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Natural Antioxidants Reduce Near-UV Induced Opacification and Filamentous Actin Damage in Dogfish (*Mustelus canis*) Lenses

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We have shown that near-UV radiation, predominantly in the wavelength range of 320 to 400 nm (near UV_A), degrades F-actin, both in cells and in extracts of muscle (1, 2). In this study, the ability of several natural antioxidants to protect lens epithelial cell F-actin from UV degradation was studied.

Low levels of natural antioxidants were added to incubation media (elasmobranch Ringer's solutions) in which fresh dogfish (*Mustelus canis*) lenses were maintained in the presence and absence of near-UV_A radiation for 14 h. At an irradiance of 1.5 mW/cm^2 the lenses received 63 J/cm^2 of UV-A energy.

Slit-lamp photographs of unexposed control lenses (Fig. 1a), of UV-exposed lenses without (Fig. 1b) or with (Fig. 1c) A, C, E mixture are shown. Without UV exposure, the lenses remained clear, but with UV exposure they developed superficial cortical opacities (see Fig. 1). The presence of 10 μM α-tocopherol (E) or a mixture of 10 μM of E plus 10 μM β-carotene (A) plus 0.1 mM ascorbic acid (C) partially protected against opacification. Only the unprotected UV-exposed lenses developed an opacity.

F-actin normally appears in lens epithelial cells as stress fibers. Fluorescence microscopy of epithelial cells stained with rhodamine-phalloidin dye (a specific reactant for polymerized actin) revealed a large diminution of F-actin fluorescence after the cells had been exposed to near-UV_A (Fig. 1e) as compared to the unexposed control epithelia (Fig. 1d). Those lens epithelial cells exposed to UV with A, C, E added (Fig. 1g) retained their normal actin filaments. Surprisingly, the lens epithelial cells that were kept in the dark and also given A, C, E (Fig. 1f) exhibited greater F-actin fluorescence than the controls (Fig. 1d). This observation requires further investigation.

To quantitate the decrease of F-actin in these cells, a modified chemical assay for F-actin (3) was applied to extracts of lens-capsule epithelium from control or UV-exposed lenses, with or without A, C, E added to the medium. The results of the chemical assay used to determine polymerized actin was applied to 14,000 rpm supernates of lens capsule epithelia homogenized in polymerizing buffer [$MgCl_2$ (2 mM), KCl (50 mM), and ATP (2 mM)]. The 580 nm emissions due to 550 nm excitation were measured with an Aminco-Bowman fluorometer.

The values of F-actin fluorescence of dogfish lens capsule epithelia were determined in several experiments. UV-exposure diminished the F-actin by about 30 to 40% when no additives were present, but the control values were retained when A, C, E was added to the incubation medium. E alone also protected against a decrease of F-actin due to UV exposure. A and C were not protective. When supernatants of frozen lens capsule-epithelial homogenates were similarly UV-irradiated, a 43% loss of F-actin was observed.

The data support the following conclusions: Near-UV_A radiation at lower than solar levels leads to superficial dogfish lens opacities *in vitro*. These opacities are diminished when α-tocopherol (E), or a combination of E and β-carotene (A) and ascorbic acid (C), are present in the medium. A or C alone did not protect F-actin from degradation.

This radiation resulted in histologically observable damage to