Activation of sea urchin eggs by ionomycin is dependent on extracellular calcium.

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Introduction

Calcium ion concentrations increase inside an egg after fertilisation by a sperm. This leads to an envelope forming around the egg, to ensure only the one sperm nucleus gets into the cell.
Thapsigargin is a drug that blocks the calcium pump that collects the ions from the cytoplasm and puts it into storage, but has no effect on the channels that leak it back out, resulting in the calcium stores emptying.
Ionomycin is a calcium ion carrier that inserts itself into the plasma membrane of cells, allowing calcium ion influx into the cell.
These two drugs give similar fluorescence response curves to fertilisation of eggs, yet thapsigargin never results in envelope formation, and ionomycin only does with calcium ions present outside the cell.

Aims and objectives

- Investigate the relationship between thapsigargin and ionomycin with fertilisation envelope formation
- Investigate the effect on the presence of extracellular calcium on this relationship

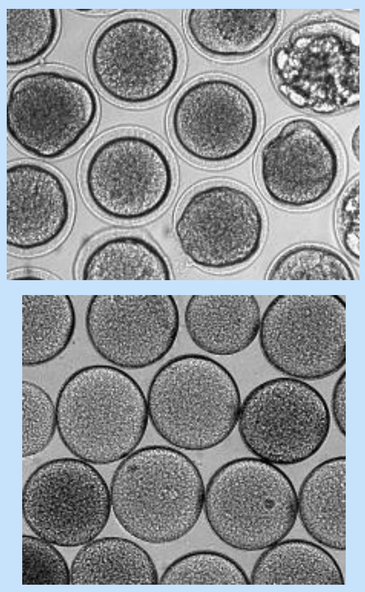

Figure 1. Egg cells with (top) and without (bottom) envelopes.

Methodology

- Microinjection was done with calcium green, with injections at 2μM. Problems with injection led to the use of many different needle types, with varying thicknesses and taper lengths.
- Eggs had to be washed in calcium free sea water by gentle centrifugation for the calcium free experiments. Calcium added back was made up to the same concentration of calcium in the artificial sea water used for the other experiments.
- Data for the graphs was recorded by the fluorescence imaging software MetaFluor, and images of egg envelopes were taken using MetaMorph.

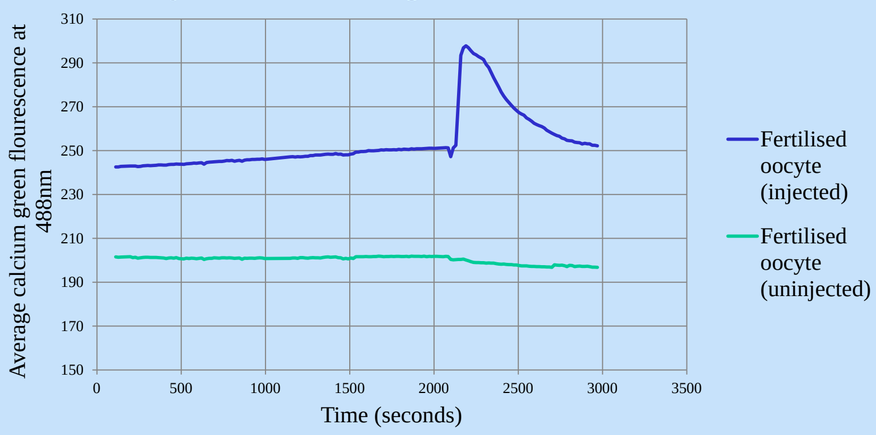

Figure 2. Graphs to demonstrate calcium ion concentration increase in egg cells, done by measuring fluorescence of a dye known as calcium green, which fluoresces more intensely as calcium ion concentration increases. Top graph shows the difference in fluorescence in calcium green injected cells versus non injected cells when both are fertilised. Bottom graph shows the differences in fluorescence between fertilised and non fertilised eggs when both are microinjected. All fertilised cells produced an envelope, the unfertilised egg did not.
Note: different starting points of fluorescence is due to varying concentrations of dye injected, due to factors such as using different needles, and successfulness of the injections.

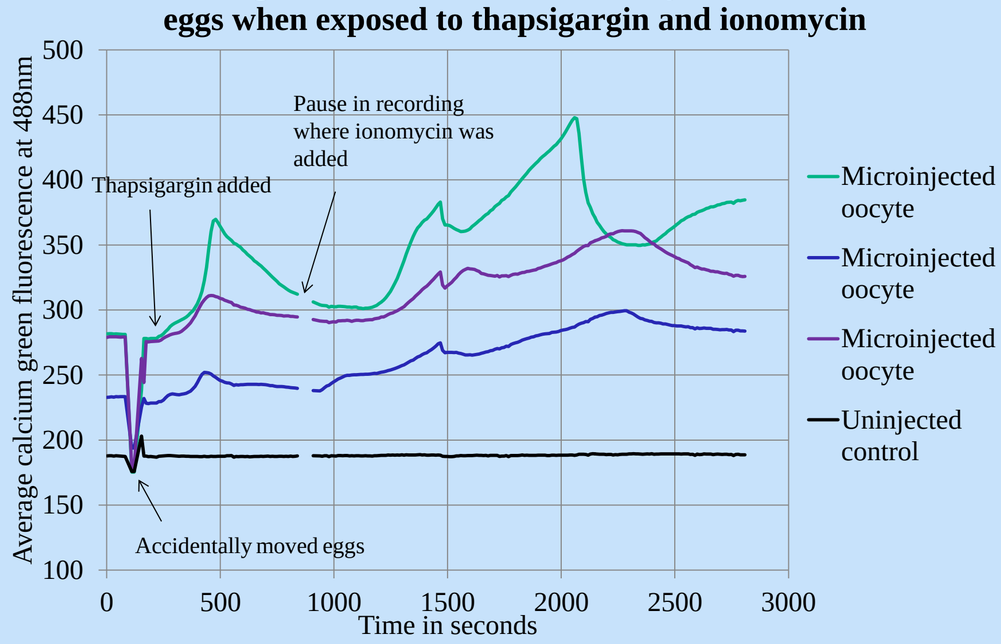

Figure 4. Graph to prove fertilisation envelopes can still be raised in an egg cell treated with thapsigargin by adding ionomycin. No envelopes were present until after the ionomycin was added.

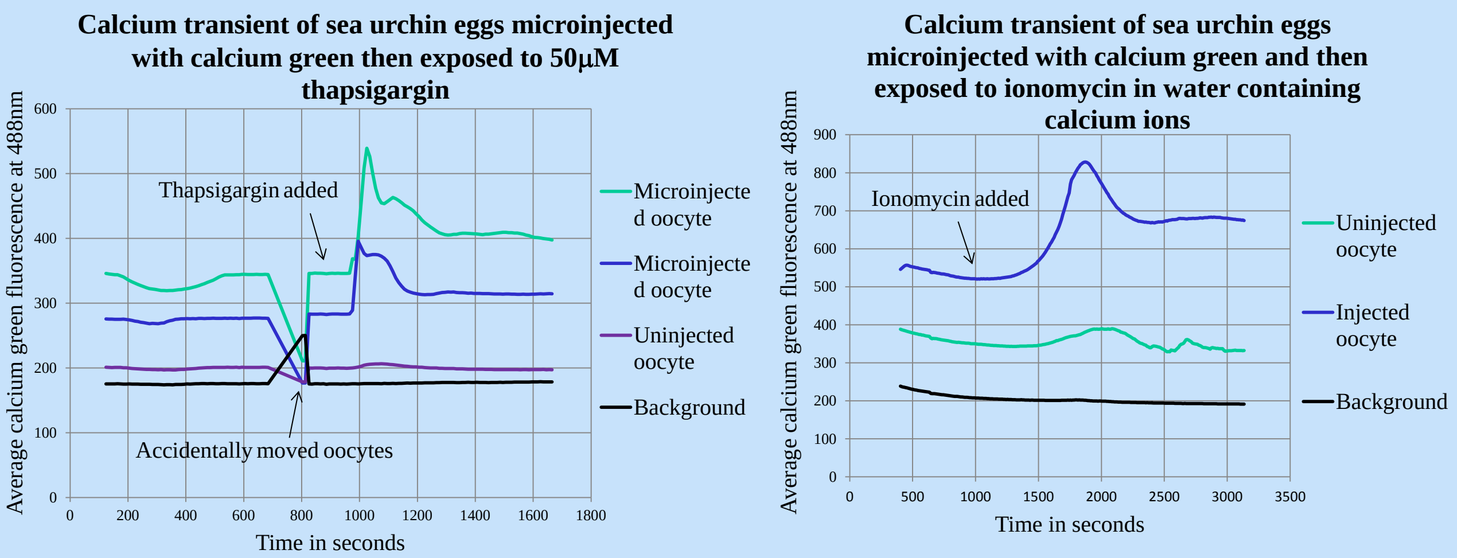

Figure 3. Graphs showing calcium increases with exposure to thapsigargin and ionomycin. Eggs exposed to thapsigargin did not raise an envelope, but those exposed to ionomycin did.

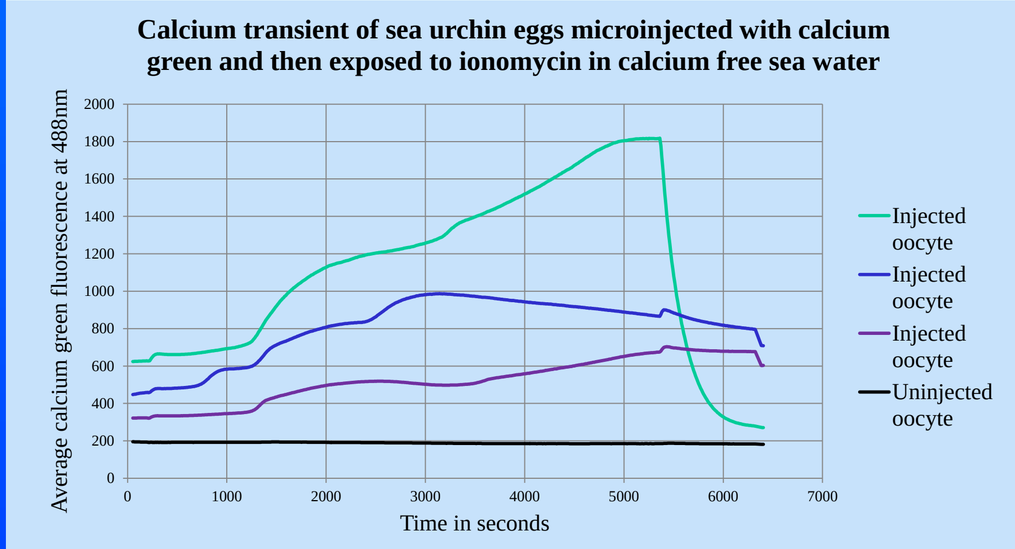

Figure 5. Graph showing no envelope being raised while eggs were kept in calcium ion free sea water, though results do show high calcium concentration increase. The only place this could have come from would be the cell's calcium stores. As no envelopes were raised, the lack of external calcium showed the same results as thapsigargin.

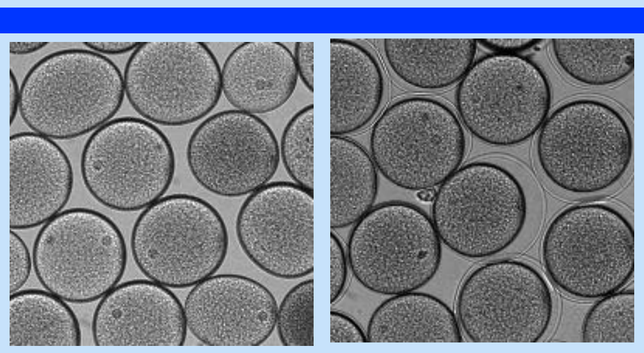

Figure 6. Eggs kept in calcium free sea water exposed to ionomycin raised no envelopes, but adding calcium back into the water caused envelope formation. Thapsigargin showed no envelope before nor after calcium add-back.

Discussion

Evidence suggests that entry of external calcium ions is the key factor in envelope formation when treated with ionomycin.
A weakness of the project was the inability to correctly standardise calcium green injection, meaning there can be no accurate comparison of the quantities of calcium ion concentrations, and comparison of the rises from the calcium starting point has to suffice. However, results are consistent with Figure 5, which has the greatest calcium rise of all the experiments, showing that fertilisation envelopes cannot be raised by release of internal calcium by either thapsigargin or ionomycin.
However, external calcium isn't always necessary; it is known that the ionophore A21837 manages to activate sea urchin eggs in calcium free sea water[1], it is possible this mechanism bypasses any process the egg has that stops the same happening with ionomycin or thapsigargin. Further research would be required.

Acknowledgements

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Project and poster by Justin Engelbert

[1]Biological Sciences: Cell Biology:Richard A. Steinhardt and David Epel; Activation of Sea-Urchin Eggs by a Calcium Ionophore; PNAS 1974 71 (5) 1915-1919