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Intracellular pH Measurements During Fertilization of Surf Clam (*Spisula solidissima*) Oocytes

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Previous work indicates that surf clam (*Spisula solidissima*) oocytes undergo increases in both intracellular Ca^{2+} and pH upon activation. However, direct measurements of both ionic responses in live oocytes, following fertilization, are still lacking. We measured intracellular pH (ph_i) in 2′,7′-bis(carboxyethyl)-5(6) carboxyfluorescein (BCECF)-loaded oocytes monitored with a Zeiss Attofluor digital fluorescence imaging microscope.

Oocytes were loaded with BCECF-AM (5 µM) for 15 min and washed in MBL artificial seawater containing 5 mM TRIS-HCl, pH 8.2. Groups of oocytes were put on polylysine-coated coverslips attached to petri dishes and their fluorescence signals at 520 nm were periodically (6-s intervals) measured for excitation wavelengths set at 430 and 488 nm. Fluorescence intensity ratios (488/430, FlRs) were thus monitored as indicators of pH_i.

The addition of 5 mM ammonia induced immediate large increases in FlRs that rapidly, within seconds, reached a new stable level and then decreased slowly with time (Fig. 1). The addition of sperm to oocytes caused slow increases in FlRs, after a varying lag period (1-2 min). FlRs in fertilized oocytes steadily increased over a 10-min period, after which they remained stable for extended periods of time (>20 min, Fig. 1). The addition of 50 mM KCl to oocytes caused immediate increases in FlRs that leveled off within 5 min to values similar to those of fertilized oocytes (Fig. 1). The pH shift in fertilized or KCl-activated oocytes was completed before germinal vesicle breakdown (GVBD) and amounted to about half of the upward shift seen after ammonia addition that did not induce GVBD. According to an *in vitro* calibration curve, resting pH of surf clam oocytes is about 6.82. Ten minutes after fertilization, the ph_i goes up to 7.24 for an average rise (ΔpH_i) estimated as 0.42 pH units. KCl caused a similar rise in pH_i (ΔpH_i = 0.33), whereas 5 mM ammonia raised it initially to much

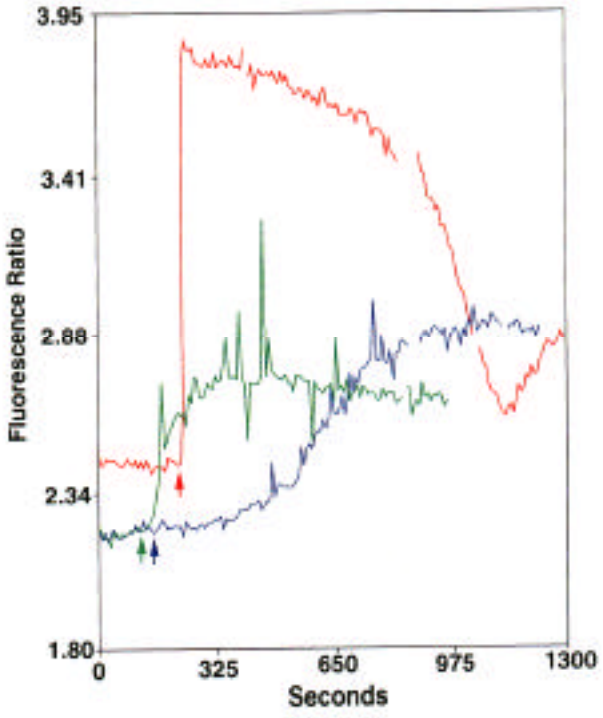

Figure 1. *Intracellular pH changes measured in BCECF-loaded surf clam (*Spisula solidissima*) oocytes: the effects of fertilization and additions of KCl or ammonia. Red line. Ammonia (5 mM) was added at the arrow No gerrninal vesic/e breakdown (GVBD) occurred during the experiment. Average readings From 6 oocytes. Blue line. Normal fertilization with sperm added at the arrow. All oocytes underwent GVBD. Average readings from nine oocytes. Green line: Artificial activation by KCl (50 mM) added at the arrow. All oocytes underwent GVBD. Average readings from nine oocytes.*

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higher values (ApH_i = 0.78). Nevertheless, it is known that ammonia concentrations higher than 10 mM are required to induce GVBD in this species (4), which strongly suggests that the activating effect of such high ammonia concentrations is not solely related to its capacity to raise pH.

On the basis of these experiments, it appears that an increase of pHj occurs after fertilization or artiiicial activation of surf clam oocytes. Apparently, however, this upward pl I shift is not sufficient for the onset of GVBD.

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Literature Cited

1. **Allen, R. D. 1953.** *Biol. Bull.* **105**: 213-239.
2. **Ii, I., and L.I. Rebhun. 1979.** *Dev. Biol.* **72**: 195-200.
3. **Ii, I., and L. I. Rebhun. 1977.** *Cell Motil. Cytoskel.* **10**: 344.
4. **Dubé, F. 1988.** *Dev. Biol.* **126**: 233-241.