

Reference: Biol. Bull. 191: 280-281. (October, 1996)

## Effects of Altering $pH_i$ and $pH_o$ on the Activation of *Chaetopterus* Eggs

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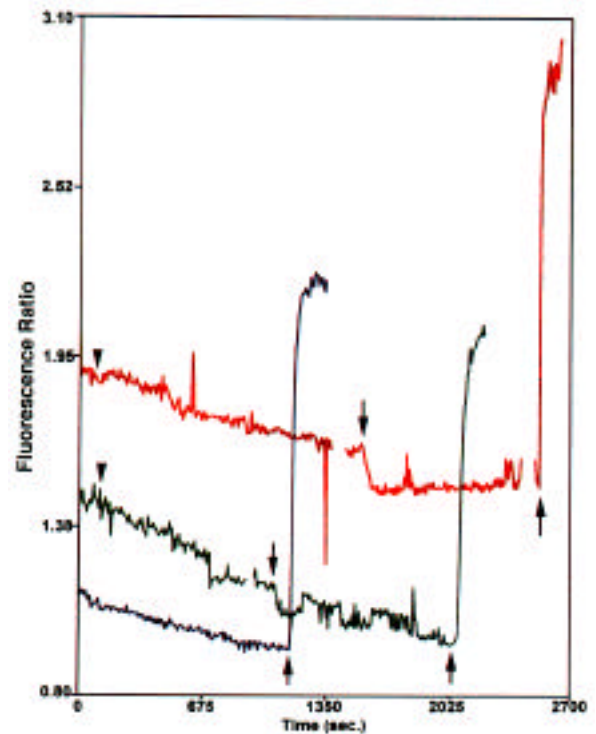
*Chaetopterus* eggs are shed at prophase of meiosis I and undergo germinal vesicle breakdown (GVBD) upon contact with natural seawater (NSW). The initiating agent in NSW is unknown, but it must be a trace component because no artificial seawater formula is able to induce GVBD. After GVBD they form a spindle and arrest at metaphase until they are fertilized or artificially activated. If fertilized, they complete meiosis, begin cleavage and develop into larvae; if artificially activated with 50-100 mM excess  $K^+$ , they complete meiosis and undergo differentiation without cleavage (2).

Metabolic activation after fertilization of sea urchin eggs involves increases in cytoplasmic free  $Ca^{2+}$  and pH ( $pH_i$ ) (3, 4). While increases in intracellular free  $Ca^{2+}$  seem to be universally involved in egg activation, the role of  $pH_i$  is controversial. In *Spisula* the  $pH_i$  increases after fertilization, but this increase is evidently not an important signal in the initiation of development (5, 6). Furthermore, the pH does not increase in starfish eggs at maturation or fertilization (7, 8). Accordingly, we have investigated the possible role of  $pH_i$  changes at oocyte maturation and egg activation in *Chaetopterus* by two approaches. We tested the effects of reduced extracellular pH ( $pH_o$ ) and  $NH_4Cl$  on GVBD and egg activation, and we measured changes in intracellular pH using fluorescence microscopy.

We performed several experiments to test the effects of changing  $pH_o$  and  $pH_i$  on GVBD and egg activation. We first tested the ability of NSW (buffered with 10 mM each of HEPES and PIPES) to induce GVBD at pH 6.0, 6.5, 7.0, 7.5, 8.0, and 8.2 (the normal pH of seawater). GVBD was normal at all  $pH_o$  tested. In NSW buffered at pH 6.0 with 10 mM Na acetate, germinal

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**Figure 1.** Fluorescence ratio (430 nm and 488 nm excitation, 520 nm emission) of 2',7'-bis-(2-carboxyethyl)-5-(and-6)-carboxyfluorescein (BCECF) in *Chaetopterus* oocytes during GVBD and egg activation. Oocytes were loaded with 5  $\mu M$  BCECF-A M (a cell-permeant acetoxymethyl ester of BCECF) for 15 min at room temperature and washed twice with CaFASW



and attached to polylysine-coated cover slips for observation by epifluorescence. Downward traces indicate acidification of the cytoplasm upward traces indicate alkalinization. The small peaks are machine "noise." The red (5 oocytes) and green (2 oocytes) traces show the results from two different experiments. The blue trace represents a control experiment in which unstimulated oocytes were left in CaFASW. Arrowheads indicate the time of addition of natural seawater to trigger GVBD downward-pointing arrows indicate the time of addition of 100 mM KCl to activate the eggs upward-pointing arrows indicate at the time of addition of 10 mM  $NH_4Cl$  which was added at the end of each experiment to ensure that alkalinization of the cytoplasm could have been detected had it occurred

vesicles disappeared, but spindles failed to form, and therefore, this is not a true progression to M phase. Next we treated oocytes with  $\text{NH}_4\text{Cl}$ , pH 8.2, which directly increases  $\text{pH}_i$  (9). However, concentrations of  $\text{NH}_4\text{Cl}$  up to 10 mM in artificial seawater failed to induce GVBD. At 20 mM  $\text{NH}_4\text{Cl}$  germinal vesicles disappeared, but, as with acetate buffered seawater, spindles failed to form. Third, we tested  $\text{NH}_4\text{Cl}$  on metaphase-arrested oocytes. Concentrations up to 0.5 mM had no effect. Higher concentrations caused elevation of some fertilization envelopes in a dose-dependent manner. In *Chaetopterus* oocytes fertilization envelope elevation results from cortical microvillar elongation (10). At 10 mM  $\text{NH}_4\text{Cl}$ , some oocytes became ameoboid, indicating the onset of pseudocleavage, a sign of differentiation without cleavage; at 20 mM, approximately 20% of the oocytes formed protrusions at the animal pole, which resembled attempts at polar body formation. By 90 min after activation, many oocytes in 20 mM  $\text{NH}_4\text{Cl}$  had polar bodies, and some were ameoboid; a few in 10 mM  $\text{NH}_4\text{Cl}$  also had polar bodies and were ameoboid. However, at these high concentrations,  $\text{NH}_4\text{Cl}$  raises the pH to unphysiological levels (Fig. 1) and may have other effects as well (11), so it would be unwise to attribute this egg activation solely to increased  $\text{pH}_i$ .

We used the Attofluor digital microscope to perform experiments to examine whether the  $\text{pH}_i$  changes during GVBD or egg activation (Fig. 1).  $\text{pH}_i$  underwent a slow downward drift during GVBD. We do not believe that this represents acidification of the cytoplasm associated with GVBD, as a similar drift was observed in oocytes not undergoing GVBD. Addition of KCl to a final 100 mM activated eggs as evidenced by polar body formation. This involved a brief, more rapid, decrease in followed by stabilization of  $\text{pH}_i$ . Addition of  $\text{NH}_4\text{Cl}$ , pH 8.2, to a final 10 mM resulted in a rapid and substantial alkalinization of the cytoplasm. As shown above, this treatment did not elicit GVBD in prophase-arrested oocytes and only weakly elicited

polar body formation in metaphase-arrested oocytes.

We conclude that the  $\text{pH}_i$  changes little, if at all, during GVBD and after egg activation in this organism and that pH has no obligatory role in GVBD or egg activation.

We thank Dr. Peter J. S. Smith for access to the Attofluor microscope, supported by NCRR (P4 I RR0 1395). W.R.E. was supported by grants from the NIH (GM080 16) and the Council for Tobacco Research, USA, Inc. (#3378). F.D. was supported by an NSERC-Canada grant.

#### Literature Cited

1. Ikegami, S., T. S. Okada, and S. S. Koide. 1976. *Dev. Growth Differ.* **18**: 33-43.
2. Lillie, F. R. 1902. *Wilhelm Roux Arch. Entw. Org.* **14**: 477-499.
3. Epel, D. 1990. *Cell Differ. Dev.* **29**: 1 - 12.
4. Jaffe, I. F. 1985. Pp. 127- 165 in *The Fertilization Response of the Egg*. C. B. Metz and A. Monroy, eds. Academic Press, New York.
5. Dubé, F. 1988. *Dev. Biol.* **126**: 233-241.
6. Dubé, F., and W. R. Eckberg. 1996. *Biol. Bull.* **191**: 279-280.
7. Johnson, C. 11., and D. Epel. 1982. *Dev. Biol.* **92**: 461 -469.
8. Peaucellier, G., A. Picard, J. J. Robert, J. P. Capony, and J. C. Labbe. 1988. *Exp. Cell Res.* **174**: 71-88.
9. Epel, D., and F. Dubé. 1987. Pp. 363-393 in *Control of Animal Cell Proliferation*. A. L. Boynton and H. L. Leffert, eds. Academic Press, New York.
10. Eckberg, W. R., and Y. 11. Kang. 1981. *Differentiation* **19**: 154-160.
11. Dubé, F., and D. Epel. 1986. *Exp. Cell. Res.* **162**: 191 -204.