

**Figure 1.** (A) Schematic representation of the device used to collect the data (ap = animal pole of the egg). (B) Uninterrupted recording (i.e., no video images taken) of aequorin-generated light emission from an albino egg bathed in APW containing calcium. In cases where photon collection was interrupted and video images were taken in order to establish a temporal correlation between calcium oscillations and cleavage, it was

*n* = 5) illustrates, additional calcium spikes, superimposed on the overall oscillations, were consistently detected while eggs were developing in calcium-free medium (APW minus  $\text{CaCl}_2$  + 1 mM EGTA). Recording from the animal hemisphere alone, Keating *et al.* (2) did not detect such transients from albinos developing in calcium-free medium. Kubota *et al.* (1) did not report any data from the vegetal hemisphere under these particular (i.e., calcium-free) conditions. We suggest, therefore, that such superimposed spikes seen through the first few cell cycles represent early calcium-related developmental events specific to the vegetal hemisphere. The spatial nature and developmental significance of these calcium transients are currently under investigation.

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#### Literature Cited

1. Shimomura, O., S. Inouye, B. Musicki, and Y. Kishi. 1990. *Biochem. J.* 270: 309-312.
2. Kubota, H. Y., Y. Yoshimoto, and Y. Hiramoto. 1993. *Dev. Biol.* 160: 512-518.
3. Keating, T. J., J. R. Cork, and K. R. Robinson. 1994. *J. Cell Sci.* (in press).
4. Miller, A. L., E. Karplus, and L. F. Jaffe. 1994. *Methods in Cell Biol.* 40: 305-338.

clear that furrows appeared on the animal hemisphere at the bottom of the calcium oscillation (data not shown). The small arrowheads on both (B) and (C) indicate when furrows would be expected to appear on the animal hemispheres of the two embryos generating these traces. (C) Uninterrupted recording of an albino egg bathed in calcium-free APW. Asterisks mark the unexplained vegetal hemisphere calcium transients.

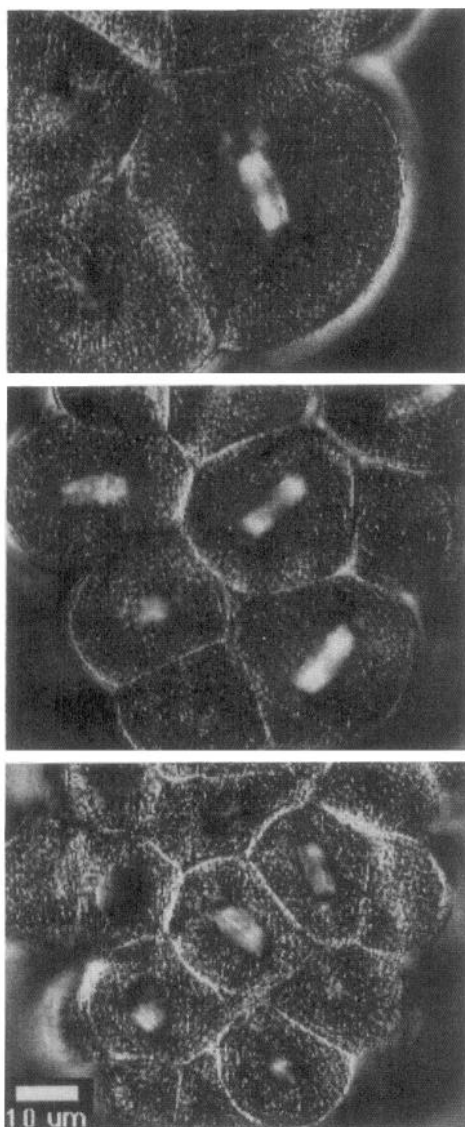
Reference: *Biol. Bull.* 187: 240-241. (October, 1994)

### Mitosis, Cleavage, and Development of Highly Compressed Sea Urchin (*Lytechinus variegatus*) Zygotes Fabrice Roegiers, Phong Tran, and Shinya Inoué (Marine Biological Laboratory)

The position of the mitotic spindle determines the cleavage plane of the cell undergoing mitosis. This in turn defines whether the cell will cleave equally or unequally. In the sea urchin, spindle position and orientation are important for formation of the micromeres, which occurs when the vegetal nucleus or spindle migrates to the vegetal cortex of the lower blastomeres at the eight-cell stage. Earlier studies have shown that flattening of the embryos at the two- and four-cell stages perturbs micromere formation in most embryos (1, 2). It is thought that the spindle is unable to move freely in flattened blastomeres and thus cannot reach its target site at the vegetal cortex. In the few cases where micromeres did form, this target zone appeared to lie in a plane

perpendicular to the compression. In our study, we observed embryos compressed in an agarose sandwich with polarized light microscopy to visualize both spindle formation and orientation, and cytokinesis. By coupling this methodology to time-lapse video microscopy we were able to observe cleavage over long periods.

Eggs of *Lytechinus variegatus* were fertilized in 1 mM aminotriazole to prevent hardening of the fertilization envelope and were allowed to reach the two-cell stage. The fertilization envelope was then removed by filtration through a 74- $\mu\text{m}$  Nytex filter and the embryos were placed in 70%/30%  $\text{Ca}^{2+}$ -free ASW/ASW (artificial seawater). The embryos were then mechanically



**Figure 1.** Sequential computed images of a compressed *Lytechinus variegatus* zygote at 8-, 16-, and 32-cell stages. All spindles in side view, regardless of orientation, show up as white areas of high birefringence. The metaphase plate coincides with the plane of cytokinesis. These images were generated using Oldenbourg's new pol-scope (5). Four images were taken at different compensator angles; from these a computed image with a pixel brightness proportional to the sample birefringence regardless of specimen orientation was rapidly and automatically calculated.

compressed between two thin sheets of agarose (3) or between a sheet of agarose and a coverslip. Compression down to 20  $\mu\text{m}$  was achieved. These compressed embryos were then mounted in a micro-drop chamber for observation with either a polarized

light microscope (4) or a novel pol-scope (5). Development was followed by time-lapse video. With the new pol-scope we were able to visualize spindles in any orientation in the plane of focus. This allowed us to predict the cleavage pattern of the embryos more precisely (Fig. 1).

In embryos that were compressed between two sheets of agarose, we observed that highly flattened embryos continued to cleave as a monolayer. Overnight these embryos reached several thousand cells and after 15 h of development, the cells had spread out over several fields of view. In one such embryo, we did observe the formation of micromeres at the 16- and 32-cell stages. These embryos showed no evidence of differentiated structures (ciliated cells, spicules, etc.).

Embryos compressed between coverslip and agarose sheet did not remain highly compressed. These embryos began forming a second layer of cells at the 5th cleavage (32 cell stage). In these embryos, epithelial differentiation, ciliogenesis, and perhaps even primordial spicule formation were observed.

These results show that highly compressed embryos can continue to divide despite a forced repositioning of the spindle. We have reason to believe that the commonly observed inability of eggs to proceed beyond one or two divisions when sandwiched between slide and coverslip is due to depletion of the oxygen supply; it is clearly not due to deformation from compression. In this connection, Ziegler has managed to grow compressed eggs to cleave up to the 64-cell stage by perfusing the slide constantly with fresh seawater (2).

We plan to use this technique, in combination with 4-D polarized light microscopy, to observe the behavior of spindles in compressed blastomeres in order to study the precise behavior of the spindle preceding a critical unequal cleavage and to explore the localized distribution of the vegetal pole determinant.

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## Literature Cited

1. Dan, K., 1987. *Dev. Growth Diff.* 29(5): 503-515.
2. Morgan, T. H. 1927. *Experimental Embryology*. Columbia University Press. Pp. 468-472.
3. Fukui, Y., S. Yumura, T. Yumura, and H. Mori. 1986. *Methods Enzymol.* 134: 573-580.
4. Inoué, S. 1986. *Video Microscopy*. Plenum Press. Pp. 493-497.
5. Mei, G., and R. Oldenbourg. 1994. *Proceedings of the Conference on Polarization Analysis and Measurement II, SPIE International Symposium* (in press).