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TRACKING CELLS IN EMBRYOS

Stephen W. Carmichael[1], Mayo Clinic

Ever since the first embryo was put under a microscope, scientists have been trying to determine which embryonic cells form which structures in the mature organism. Classic methods were to kill (ablate) certain cells and see what structures were malformed or missing when the animal developed. More recently, cells have been labeled with visible tags and the tagged cells traced through development. One problem is that the tag gets diluted by half each time a labeled cell divides. A new labeling method that shows a lot of promise has been demonstrated by David Piston, Robert Summers, Susan Knobel, and John Morrill.[2]

Using the sea urchin *Lytechinus variegatus* as a model, Piston *et al.* injected fertilized eggs with a specially-prepared fluorescent dye. Fluorescein was surrounded with a complex of dextran molecules so that the fluorescein was "caged." About 10 picoliters of the caged fluorescein was delivered to an egg before the first cell division. When the embryo was exposed to ultraviolet light, the fluorescein was released from its dextran cage and all of the embryonic cells fluoresced. This demonstrated that the fluorescent tag was evenly distributed by this method and that it could be uncaged by a specific treatment with light. Additional control experiments showed that the dextran-caged fluorescein did not interfere with normal development.

Using a customized microscope setup, immobilized embryos could be visualized by confocal microscopy. Selected cells of the embryo could be brought within the focal volume of the two-photon excitation pathway. After the cells were selected and targeted, the cells were hit with the Ti:Sapphire laser using a custom-built mirror drive circuit. This resulted in the fluorescein being released from its dextran cage only within the targeted cells. The location of the selected cells within the intact embryo could be followed with confocal and Nomarski differential interference contrast microscopy.

An objective of this study was to determine where the gut of this animal came from. Although this problem has been investigated for about a century, the techniques have not existed to give a clear answer. Using their newly-developed technique, Piston *et al.* found that cells from the ectodermal-endodermal junction (this is the blastoporal margin at the stage when the cells were marked) moved to a position deep within the early tubular gut. The total process of gastrulation was found to take longer than previously described. Relatively late in gut development, cells that would eventually form the intestine were shown to still be in the lateral body wall. Observations from this study suggested that final determination and differentiation of the cells of the gut wall are put on hold until all of the cells are in place and the gut tube is functional. This is in agreement with a newly developing model of sea urchin gastrulation.

Whereas this is an important study for the embryologist working with sea urchins, the methods developed to examine this model have even more far-reaching implications. The ability to mark specific cells, then follow them in the living embryo to determine their fate will be useful in a broad range of studies, both in invertebrate and vertebrate embryos. This could be the best method yet for tracking cells in embryos!

1. The author gratefully acknowledges Dr. David Piston for reviewing this article.

2. Piston, D.W., R.G. Summers, S.M. Knobel, and J.B. Morrell, Characterization of involution during sea urchin gastrulation using two-photon excited photorelease and confocal microscopy, Microsc. Microanal. 4-404-414, 1998.

Front Cover Image
Orientation Imaging Map Of Aluminum Thin Film

The 560 nm x 560 nm cover image of highly textured aluminum thin film was obtained by collecting multiple dark field images under computer control of the tilt and rotation of the TEM beam. By tracking bright up events in the dark field images, it is possible to characterize the crystallographic orientation of the individual crystallites in the aggregate image. In this image blue grains indicate that the <110> crystal direction is closely aligned with the vertical direction whereas red grains are directed nearly 30° away from the vertical.

Image compliments of TSL, Inc. For further information on this subject, see article on page 12 of this issue.

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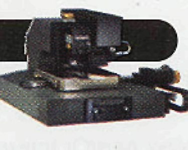

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