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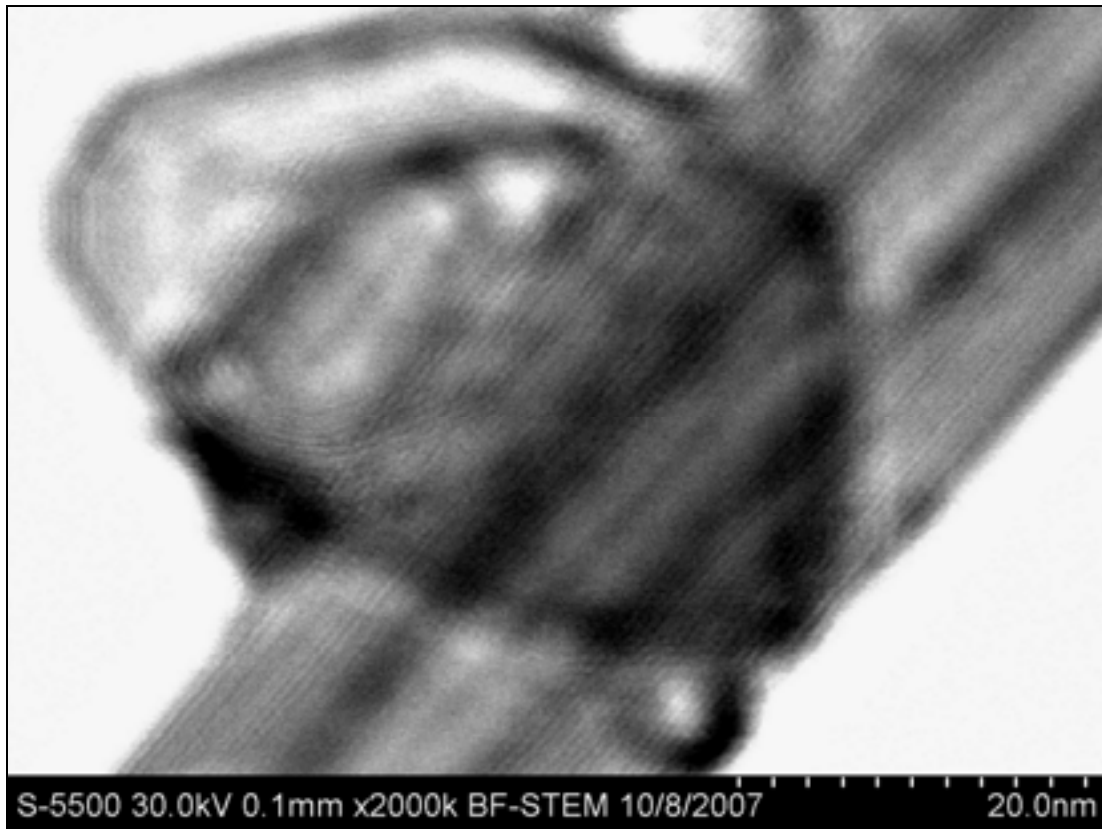
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# Establishing the Initial Embryonic Axis

Stephen W. Carmichael and Gary C. Schoenwolf<sup>1</sup>

*Mayo Clinic and the University of Utah*

In the mammalian embryo, the first axis to appear is at the time of the fifth cell division when the inner cell mass (ICM) becomes visible. The localization of the ICM on one side of a cavity formed within the cluster of dividing cells marks the embryonic-abembryonic (E-Ab) axis. This name derives from the fact the most of the embryo will develop from the ICM, whereas other tissues (the placenta, etc.) will develop from the other cells. There has been a long-standing controversy as to what determines the mammalian E-Ab axis; is the information inherently in the zygote, or is it determined after several cell divisions? In an elegant series of studies whereby dividing cells were labeled using new molecular genetic tools and then carefully followed during development, Yoko Kurotaki, Kohei Hatta, Kazuki Nakao, Yo-ichi Nabeshima, and Toshihiko Fujimori have provided an answer in a mouse model.<sup>2</sup>

Kurotaki *et al.* focused on the preimplantation stage (the early period of development before the embryo becomes implanted in the uterus) of mouse development to reveal the factors that establish the E-Ab axis. First they performed studies *in vitro*. They generated a transgenic mouse in which all of the cell nuclei express a form of green fluorescent protein and then traced the fate of the labeled cells every ten minutes using time-lapse, fluorescence and bright-field microscopy. Fluorescent images were captured at 5 micron steps in the Z axis so that a 3-dimensional map of the dividing cells could be generated. They examined 124 embryos and most (87%) developed into a ball of cells with a fluid-filled center.

Another structure that was observed was the zona pellucida (ZP), a flexible, shell-like membrane of glycoproteins that surrounds the early embryo. Kurotaki *et al.* found that it plays a role in shaping the embryo. For example, they found that the position of the ZP relative to the culture

plate remained fixed, whereas the embryo rotated within the ZP. In other experiments, they chemically dissolved the ZP and showed that the shape of the unconstrained embryo differed from the embryo within a ZP. The ZP conferred an ellipsoidal shape to the early embryo.

In another series of impressive experiments, Kurotaki *et al.* were able to extend their studies to an *in vivo* murine model. Another strain of transgenic mice was established in which all cells up to the blastocyst stage express green-red protein, a protein that under normal circumstances fluoresces green. However, when mildly irradiated with u-v-light, the protein fluoresces red. Hence, at the two-cell stage, one cell was irradiated with u-v light, allowing for the cells derived from each of the two blastomeres to be distinguishable from each other. The two-cell embryo was immediately transferred to the oviducts of receptive females. When the embryos were later recovered it was apparent that neither the green cells nor the red cells contributed exclusively to the ICM or to the abembryonic tissue. The *in vivo* experiments produced the same results as the *in vitro* time-lapse analysis. Specifically, the E-Ab axis of the embryo is formed independently of the cell lineage of the two-cell stage.

Kurotaki *et al.* used several different methods of microscopy and cell labeling to definitively answer a basic question of embryology. They showed that the first axis to form in the mammalian embryo, the E-Ab axis, is not dependent on the early origin of the cells. However, the zona pellucida does play a role in determining the ellipsoidal shape of the early embryo. The molecular mechanism of differentiation remains to be determined, but the studies of Kurotaki *et al.* will certainly be a basis for these future studies. ■

- 1 The authors gratefully acknowledge Dr. Toshihiko Fujimori for reviewing this article.
- 2 Kurotaki, Y., K. Hatta, K. Nakao, Y. Nabeshima, and T. Fujimori, Blastocyst axis is specified independently of early cell lineage but aligns with the ZP shape, *Science* 316:719-723, 2007.

## INDEX OF ARTICLES

**Establishing the Initial Embryonic Axis** .....3  
 Stephen W. Carmichael and Gary C. Schoenwolf<sup>1</sup>  
*Mayo Clinic and the <sup>1</sup>University of Utah*

**Beyond Death: Forensic Investigations of pre-Columbian Mummies from the Tarapacá Valley, Chile, Using Variable Pressure SEM and Raman Spectroscopy** .....6  
 S.V. Prikhodko, C. Fischer, R. Boytner, M. C. Lozada, M. Uribe, I. Kakoulli, UCLA, U. of Chicago, U. de Chile

**Applications of the Helium Ion Microscope** .....12  
 Larry Scipioni, Lewis Stern, and John Notte, ALIS Business Unit, Carl Zeiss SMT, Inc., Peabody, MA

**Methods of Maximizing X-ray Detection in SEMs** .....15  
 David Rohde, Patrick Camus, Thermo Fisher Scientific, Madison, WI

**Three Dimensional Imaging of Structure and Flow—Critical to Advances in Microfluidics** .....18  
 Carlos Hidrovo<sup>1</sup>, and Terence Lundy<sup>2</sup>, <sup>1</sup>Stanford University  
<sup>2</sup>Hyphenated Systems, Burlingame, CA

**Is a C<sub>z</sub> Corrector Necessary for Lorentz Vector Field Tomography?** ..... 24  
 Charudatta Phatak, Marc De Graef, Carnegie Mellon University, Pittsburgh, PA

**TEM of Bacteriophages Found in Marine Sources** .....28  
 A. Fejerman<sup>1</sup>, J. Polanco<sup>2</sup>, G. Lander<sup>3</sup>, T. Ajero Jr<sup>3</sup>, B. Carragher<sup>3</sup>, C. S. Potter,<sup>3</sup> Mount Miguel High School, Spring Valley, CA, <sup>2</sup>Hilltop High School, Chula Vista, CA, <sup>3</sup>Scripps Research Institute, La Jolla, CA

**Nanoscale Thermal Property Characterization of Automotive Polymer Coatings** .....32  
 Louis T. Germinario, Eastman Chemical Company, Kingsport, TN

**Computer-Controlled Polishing System For Preparing Multiple Pre-Fib TEM Specimens** .....38

D. J. MacMahon<sup>1</sup> and E. Raz-Moyal<sup>2</sup>, Micron Technology, Inc. and <sup>2</sup>Gatan, Inc., Manassas, VA and Pleasanton, CA

**Tungsten Filament Heating Effects** ..... 40  
 Paul Beauregard, Microscopist, Greensburg, TN

**The Database Solution to Particle-by-Particle Analysis of Mixed Mineral Dusts** ..... 44  
 B.R. Strohmeier, K.L. Bunker, K.E. Harris, R. Hoch, and R.J. Lee, RJ Lee Group, Inc., Monroeville, PA

**Table Top SEM Utilization in a High School Nanotechnology Course** ..... 48  
 Leonard, D.N., Appalachian State University, Boone, NC

**Microscopy for Children** ..... 54  
 Caroline Schooley, Project MICRO Coordinator, MSA

**Industry News** .....55

**NetNotes** .....58

**Advertiser's Index** ..... 74

## ABOUT THE COVER

Marine diatoms attached to the red algae *Polysiphonia* (100x), by Charles Krebs, of Issaquah, Washington (<http://krebsmicro.com>). This image won 4th place in the 2007 Nikon Small World photo contest. Rather than showing a diatom as a single inanimate frustule, as is often the case, this image depicts living specimens as they are found growing attached to the highly textured *Polysiphonia* alga. The sample was collected from Puget Sound, Washington and immediately prepared as a simple wet mount. The image was captured using differential interference contrast (DIC). In order to obtain extended depth-of-field, a z-stack of images from different focal planes was recorded. These were then combined (using Helicon Focus software) into a single image.