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A Gene Expression Spectacular: The Developing Drosophila Embryo

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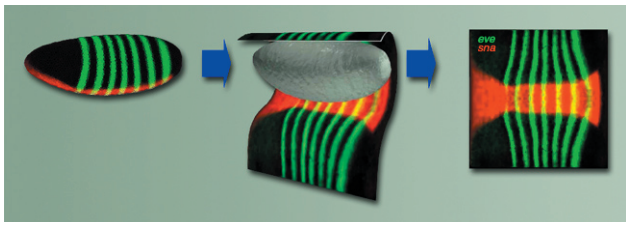
Two hours after the egg is fertilized, the embryo of *Drosophila melanogaster* reaches the blastoderm stage. The future fruit fly's development is a hotbed of activity as some 6,000 nuclei in the egg's single cell migrate to the surface and, within about half an hour, become individual new cells.



Gene expression and morphology of the Drosophila embryo, calculated at cellular resolution for the three-dimensional surface of the blastoderm, can be projected onto a surrounding cylinder and unrolled to produce a planar map. Anterior is to the left, posterior to the right. Upper and lower edges of the map represent the dorsal midline of the embryo, with the ventral midline in the center.

The blastoderm's smooth simplicity will soon be lost when the embryo begins to crease and fold into an articulated fly. Meanwhile, because its geometry is uncomplicated and the nuclei and resulting cells all lie close to the surface, the blastoderm is a natural target of investigators who want to understand how genes turn on and off and coordinate their expression during development.

The Berkeley Drosophila Transcription Network Project (BDTNP), made up of researchers from Berkeley Lab, UC Berkeley, and UC Davis, has developed methods for looking at the *Drosophila* blastoderm in three dimensions at cellular resolution, precisely measuring the expression of multiple genes as they interact to shape the fly.

"Visualizing gene expression and analyzing the morphology of an entire organism at cellular resolution has never been done before," says David Knowles of the BioImaging Group in Berkeley Lab's Life Sciences Division. The new methods of visualization have already revealed morphological and gene-expression features never seen before, overturning some long-held assumptions.

3-D revelations at cellular resolution

A striking feature of the blastoderm is the formation of "expression stripes" that encircle the embryo and move over its surface, moving from what will be the head of the organism (anterior) toward the tail (posterior). The genes giving rise to these stripes control the later segmentation of the embryo.

Prior to the work of the BDTNP, biologists studying this developmental system were limited to two-dimensional images of fixed embryos, no two of which were identical, at different stages. Gene expression could not be assigned to specific nuclei from one image to the next; the assumption arose, almost by default, that the movement of stripe patterns was due solely to gene expression and not to the motion of the nuclei.

The BDTNP collaborators also began by imaging fixed embryos, but they imaged them whole in three dimensions. Then they applied a sophisticated set of data-analysis and modeling tools. "We set up a pipeline to make and compare thousands of high-resolution microscope images of different embryos at different stages of blastoderm development," Knowles says. "For each image we stained the total DNA, which gives the location of each nucleus, plus we stained the expression patterns of two specific genes."

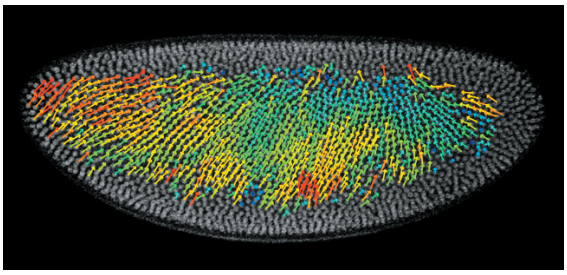
By computer analysis the three-dimensional embryo images, each some 500 megabytes in size, were converted into small, easy-to-manipulate text files called PointClouds. Each PointCloud records the position of the nuclei and the expression of two genes in and around each of them. By combining data for multiple PointClouds, the researchers could estimate the concentrations of many different gene products in each developing cell.

Mark Biggin, a biologist in the Lab's Genomics Division, says, "The developmental stage of each embryo that our PointClouds were derived from is known, but how can we say this cell in one embryo is the same as that cell in another? We devised a model—an average, statistical model—and then we tested it."

Biggin explains that with this system the researchers could predict any possible nuclear movement. "And we could separately measure the expression flow by tracking the collection of expressed genes." Unexpected features of the blastoderm immediately became apparent.

"With this new model of the embryo, we could see the previously reported shift in gene expression stripes," says Biggin, "but we also saw that the nuclei do in fact move. The gene expression patterns move partly in sync with these changes in morphology but partly independently."

The researchers also followed the development of living embryos during the blastoderm stage. The measurements were necessarily partial and less precise but confirmed the model's prediction that the nuclei move.



Stained nuclei in a single living embryo were tracked through the blastoderm stage. Red arrows indicate nuclei that moved the farthest, blue arrows those that moved least. Movement of nuclei in live embryos corresponds to predictions of the model.

The ability to model all three dimensions of the blastoderm revealed additional new information about the system. For one thing, says Knowles, "Expression of genes previously thought only to regulate development along the anterior-posterior axis"—head to tail—"also changes along the dorsal-ventral axis"—back to front.

Future Directions

Having produced the first quantitative, three-dimensional description of gene expression and morphology of the whole embryo at cellular resolution, the researchers are eager to move to the next stage of discovery.

Members of the Berkeley Drosophila Transcription Network Project include (from left) Hanchuan Peng, Angela DePace, Oliver Rübel, Jitendra Malik, Charless Fawlkes, Mark Biggin, Mike Eisen, Soile Keränen, Bernd Hamman, Cris Luengo, Damir Sudar, Gunther Weber, and David Knowles. (Image courtesy David Knowles; background shows visualization from BDTNP's PointCloudXplore program.)

"A first-order approximation of what's going on in the embryo is not good enough," Biggin says. "When you have precision right down to the level of the cell, as we now do, all you have to do is ask a question and you'll discover something. You just know you want this kind of information for the whole course of fly development, and for thousands of genes."

Knowles says, "The goal is to better understand the transcription network. We've released all of our current PointCloud data, unanalyzed, on the Berkeley Drosophila Transcription Network Project website, where anyone can download it and use it for their own research."

The BDTNP's remarkable discoveries prove the strength of uniting specialists in imaging, computer science, visualization, and biology. Their approach should lead to a better understanding of development in the fruit fly and many other organisms as well.

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