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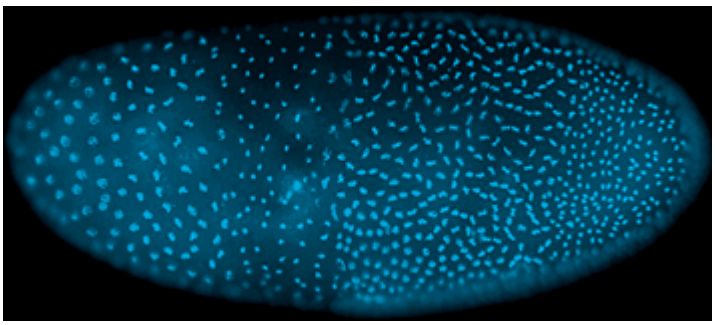
APRIL 28, 2005

Fruit Fly Development Runs Hot and Cold



The image shows the density of nuclei in a developing *Drosophila* embryo. The left half of the embryo was subjected to cool water flow; the right half to warmer water. The warmer temperature favors more rapid development, and hence the greater number of nuclei.

Using tiny flows of warm or cool water, researchers have induced one end of a fruit fly embryo to develop faster than the other. The experiments are among the first to probe the nature of the mysterious protective machinery that keeps embryonic development on an even keel, despite fluctuations in environmental conditions.

To the researchers' surprise, the experiments revealed that a compensatory mechanism enabled the embryos to develop into normal larvae despite being subjected to very unnatural conditions that the scientists thought would not favor viable development.

"The embryo is able to compensate for something that we honestly thought it would have no chance to deal with."

Nipam H. Patel

"This compensatory mechanism is absolutely remarkable," said one of the study leaders, Nipam H. Patel, a Howard Hughes Medical Institute investigator at the University of California, Berkeley. "The embryo is able to compensate for something that we honestly thought it would have no chance to deal with."

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HHMI INVESTIGATOR

Nipam H. Patel

ABSTRACT:
The Evolution of Segmentation and Body Patterning

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Illustration: William McGinnis (adapted by John Kachik)

According to the researchers, the studies were designed to ask how the compensatory mechanism works in fruit flies. The benefits of such understanding could reach far beyond the fruit fly and aid in studying crucial aspects of embryonic development in many other animals, and possibly mammals. Using this technique to induce embryos to literally run hot or cold could be combined with genetic studies to offer unique insights into the machinery that sculpts embryonic development.

The research teams, which were led by University of Chicago chemist Rustem F. Ismagilov and Patel, published their findings in the April 28, 2005, issue of the journal *Nature*.

Ismagilov, Patel and their colleagues were interested in measuring whether temperature differences could disrupt the response of the fruit fly *Drosophila* embryo to protein gradients that guide early development. For example, developing *Drosophila* embryos normally have a concentration gradient of the Bicoid protein that runs from the anterior to the posterior regions of the body. The developmental machinery in the embryo "reads" the concentration of Bicoid and translates that information into positional data that tell cells where they are located in the embryo. This information enables those cells to develop into the correct segments in the fly.

"It was known that there must be some compensatory mechanism that allows variability in this gradient to be tolerated, because studies have shown that the shape of the gradient varies considerably from embryo to embryo," said Patel. Another clue emerged from earlier experiments by HHMI investigator Eric Wieschaus at Princeton University, who showed that changing the temperature at which embryos were raised also causes considerable variation in the shape of the Bicoid gradient.

"Thus, even though we know that the embryo is reading Bicoid levels, we also know there is quite a bit of noise in the system," said Patel. "So, there must be some system to compensate for this noise and make that reading more precise. Whatever this molecular mechanism is, it has to be affected by temperature."

To get at the answers to these questions, the research team used a microfluidic system initially developed by chemists to create controlled chemical reactions of fluids. Basically, the system consists of a small chamber that can direct tiny flows of water at different temperatures past the front and rear of a developing embryo.

"The device creates a laminar flow, much like that of two rivers flowing together," said Patel. "You can actually tell by the different colored sediments of the two river flows that they don't mix right away."

In their experiments, the researchers could immediately tell that the temperature difference affected embryonic development. "We could see that the two halves of the embryos were developing at different rates," said Patel. "The first thirteen or so cell divisions during development occur in a very synchronous way throughout the embryo. We could see that in the time the cool half had gone through eleven cycles, the warm half had gone through thirteen. And surprisingly, we could see a very distinct, straight line right through the embryo defining the two halves."

As interesting as those observations were to Patel and his colleagues, the biggest surprise was yet to come. Right before their eyes, they could see the perturbed embryos fully compensating for the temperature differences and developing into normal fly larvae. "We were fully expecting that the embryo would not be able to compensate," said Patel. "We were truly amazed when the embryo did compensate."

The researchers observed that the striped segmentation patterns of the flies also developed in the normal positions, albeit more slowly in the cool half than in the warm half. "This developmental system was compensating for the perturbation that we were making on it," said Patel. "We were expecting the stripes to come on in the wrong positions in the embryo, but they were in the right position, even though their timing was off."

To determine whether there was a critical period during which the compensatory system functioned, the researchers performed experiments in which they alternated the warm and cool ends of the embryo. They began with one condition — for example warming the front and cooling the rear — then switched temperature conditions. By varying the time interval of the switching, they determined that the compensatory mechanism was "active" between 65 and 100 minutes after fertilization. "That suggests to us that it's at this critical period in development that the embryo is somehow, as it were, measuring variation and compensating for it," said Patel.

Further studies, he said, will aim to find the genes responsible for the compensatory mechanism. For example, the researchers will place mutant fly embryos lacking specific genes into the microfluidic system to identify those genes that may contribute to the compensation system..

The compensatory machinery in the fruit fly may well play important — perhaps primary — roles in embryo patterning in other animals. Early *Drosophila* development is particularly unique in how rapidly it proceeds. The system it uses to compensate for variation may be a back-up mechanism, but may be part of the primary patterning system used by other animals, he said.

According to Patel, microfluidic technology might be more broadly applied to create precise temperature differences in separate parts of the embryo to aid in exploring other aspects of embryonic development. "I think there are quite a number of biological processes — such as segmentation in vertebrate embryos — that it would be great to study by manipulating development in time and space in the same way," he said.

Image: Courtesy of Nipam H. Patel/HHMI at University of California, Berkeley.

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