Tracking the Movements That Shape an Embryo

Biologists studying a key moment in development are at last beginning to link genetic signals with the physical changes that create an embryo

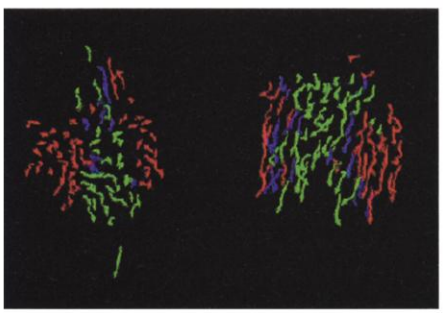
The most important time in your life, says embryologist Lewis Wolpert of University College London, "is not birth, marriage, or death, but gastrulation." Gastrulation, which in humans happens about 2 weeks after egg meets sperm, is a massive rearrangement of the embryo that transforms a relatively uniform ball of cells into a multilayered organism with a recognizable body plan. Cells stream across the embryo in a precise choreography that is strikingly similar among organisms from flies to fish to people. Although cell movements are crucial at many other times in development—and sometimes involve longer journeys (see sidebar)—if the intricate dance of gastrulation goes awry, the resulting defects are usually so catastrophic that the embryo dies.

Just what causes the cells to move and guides them to their designated places has fascinated—and frustrated—embryologists for more than a century. Developmental geneticists have fingered dozens of genes involved in controlling gastrulation, but most of them code for signaling molecules, which switch other proteins on or off. And although cell biologists have made progress in understanding how individual cells move, connections between the two fields have remained elusive.

In the past few years, however, scientists have begun to bridge the gap. They are at last linking genetic signaling cascades to molecules that actually affect the movements of gastrulation, including those that cause cells to stick together and those that promote movement.

Although there's a long way to go before scientists fully comprehend gastrulation's remodeling, these new findings are injecting a sense of optimism into the field. Knowledge of cell movement in development "is about to really explode," says University of California (UC), Berkeley, developmental biologist Richard Harland. "In a couple of years time, there's going to be a quantum difference in our understanding."

The first solid clues about the forces that drive gastrulation came a decade ago, in groundbreaking work by developmental biologist Ray Keller of the University of Virginia and his colleagues. Using fluorescent dyes and video microscopy, his team for the first time discerned the shape changes and movements that living embryonic frog cells undergo during gastrulation. Most classical embryologists had guessed that the rearrangements of gastrulation arose from cell division—that certain cells divide faster than others and change the embryo's shape. But

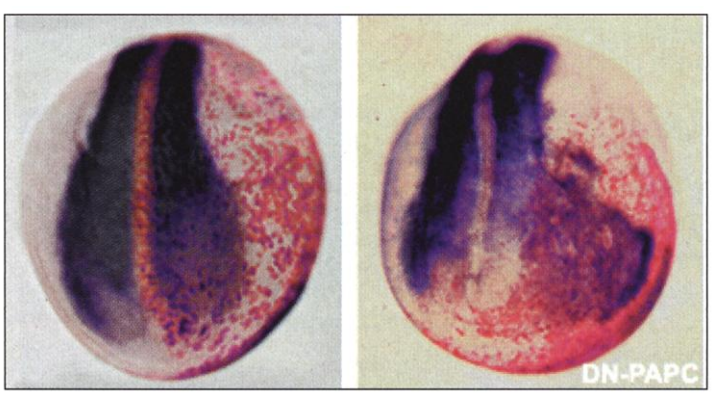


Trailblazing. Movies of zebrafish embryos show cell movement during gastrulation; arrows trace a cell's path over 20 minutes. (See movies at www.neuro.uoregon.edu/~glickman/movie2.html)

Keller and his colleagues revealed a far more active process, in which cells constantly shift places. They described a pattern of "convergent extension," in which cells converge on the embryo's midline (the precursor of the backbone) and stay there. As midline cells crowd together, they push each other toward the future head and tail, and the embryo lengthens. The cells seem to move by a process called intercalation, in which cells grab onto their neighbors and use each other as a sort of moving ladder to haul themselves toward the midline. Researchers have since observed similar cell movements in organisms from flies to mice to humans.

In frogs, gastrulation begins in the region called the "organizer," which, among other things, directs certain cells to tuck inside the relatively hollow embryo and begin to form various layers. Researchers have therefore looked among the proteins expressed in the organizer for the elusive factors that trigger cells to move. In late 1998, a team led by developmental biologist Eddy De Robertis of UC Los Angeles came up with a promising candidate: A molecule called paraxial protocadherin, or PAPC, that is expressed in both the organizer and in the cells of the developing trunk that undergo convergence and extension. PAPC, like other proteins in the protocadherin family, has a "tail" outside the cell that helps cells stick together. So the scientists expected that it would make cells stick together.

They were surprised to find that PAPC also prompts cell movement. Adding PAPC to so-called animal cap cells, which can undergo gastrulation-like movements in vitro, caused the cells to converge and extend. And when De Robertis's team injected a defective version of PAPC into one cell of a two-cell embryo, blocking the protein in half the embryo, the cells on that side failed to move toward the midline. By allowing cells to stick to one another and haul themselves forward, PAPC may help trigger convergence and extension, says De Robertis. The work, published in *Development* in December 1998, is "a very striking result," and makes a strong case that PAPC is one of the proteins crucial for prompting this unusual cell movement, agrees cell biologist Barry Gumbiner of the Sloan-Kettering Institute in New York City.



Moving violation. Cells (stained red) move to the midline in normal frog embryos *(left)*, but fail to do so when treated with a defective version of the PAPC protein (right half of embryo).

De Robertis's group, in work with Charles Kimmel of the University of Oregon, Eugene, and Sharon Amacher of UC Berkeley, was also able to add an important connection to the PAPC pathway. The researchers found that in fish, the PAPC protein showed up in the same pattern as a gene called *spadetail*, which codes for a transcription factor that turns on other genes. In embryos lacking *spadetail*, *papc* is not expressed and trunk cells fail to move toward the midline. Thus the researchers propose that *spadetail* somehow turns on PAPC, which in turn allows

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Neural Crest's Joyride Through The Embryo

While the movements of gastrulation dramatically reshape the embryo (see main text), many of the cells involved travel only a short distance. In contrast, cells known as neural crest make epic journeys, from the back of the developing brain as far as the length of the gut and the ends of developing limbs. "When it comes to cell migration, [neural crest] is king," says developmental biologist Scott Fraser of the California Institute of Technology in Pasadena. His studies of these odysseys suggest that these cells' social behavior—their interactions with their neighbors—have more sway over their final destinies than expected.

The collection of cells that make up the neural crest starts off in the precursor of the brain and spinal cord, the neural tube. Eventually the cells migrate throughout the embryo and adopt a variety of guises, becoming the bones and connective tissue of the lower face as well as the peripheral nerves that stretch throughout the body.

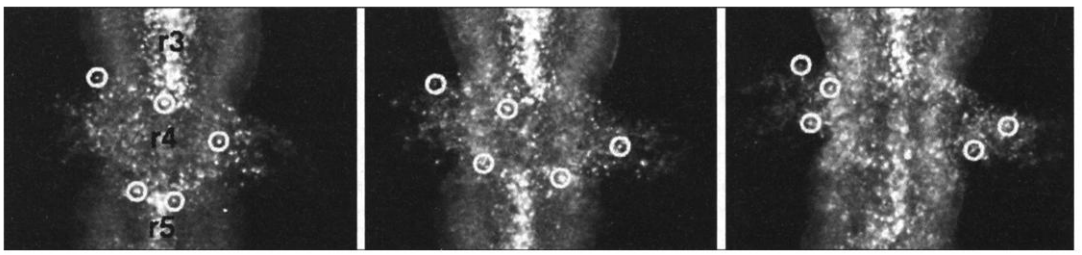
For years, scientists have followed the cells' travels by tracing the fate of cells transplanted from one embryo to another or by examining pieces of embryonic tissue kept alive in culture dishes. But Fraser and postdoc Paul Kulesa have now found a way to watch cell movements "in ovo"—in a living egg.

In one of the more ingenious uses of Teflon, the scientists cut a window into a chicken eggshell, label certain cells with fluorescent dye, and then seal the hole with a clear Teflon membrane. The membrane lets oxygen in but prevents the egg from drying out, allowing the team to observe cell movements inside the embryo for 3 to 5 days—two to three times longer than before.

Previous work had led scientists to believe that cells in the neural crest behave like workday commuters on a subway system: Before the cells set out, scientists thought, they were programmed to take regular routes to a certain destination. Cells from different parts of the neural crest seemed to group into distinct "streams" that led to specific targets—the jaw, say, or the nerves of a developing limb. But the new work, published in the March issue of *Development*, makes it "pretty clear that the cells haven't read most of those papers," says Fraser.

Instead, Kulesa and Fraser's observations suggest that these cells behave more like teenagers on a joyride, relying on cues from neighboring cells along the way to decide their route and final destination. Individual cells change routes midway and jump from one stream to the next. In some cases, cells form long filopodia—extensions of cytoplasm—that reach out to touch cells in another stream, and in a few cases the body of the cell followed. These data suggest that a cell's neighbors and the signals it detects from them may be more important than the particular genes turned on before it sets out, says Fraser.

The newfound ability to peer into a living embryo offers new insights into cells' travel habits, agrees developmental biologist David Wilkinson of the National Institute for Medical Research in London. "Now that we can really see how cells are behaving, it gives new ideas about the responses cells are giving each other," he says. "Things are less rigid than people had thought." **–G.V.**



Merging traffic. Time-lapse movies of living embryos (*left to right*) reveal cells from one migrating stream of neural crest (R5) joining another stream (R4). (See www.its.caltech.edu/~fraslab)

CREDIT: KULESA AND FRASER, *DEVELOPMENTAL BIOLOGY* 204, 327 (1998)

cells to journey to the midline. This work provides one of the first links between a transcription factor crucial to gastrulation and a molecule that changes cell behavior, Amacher says.

Before cells can move at all, they must first loosen the adhesives holding them together. Gumbiner studies a family of such protein adhesives called cadherins, which, like the related protocadherins, protrude from the cell surface and act as hooks and grapples, allowing cells to stick to each other. In experiments with cultured embryonic frog cells, he has found that the protein activin, which plays numerous roles in gastrulation, weakens the cadherins' grip and allows cells to move. Last year, Gumbiner and his colleagues developed an antibody that reactivated a protein called C-cadherin even in the presence of activin. The effect was dramatic: Although all the genes characteristic of mobile cells turned on, the cells did not move. That suggests that C-cadherin acts something like a parking brake that must be lifted to let cells move, and that it is the final molecule—the one that gets the job done—in a chain of signals.

New work on a protein called Snail shows that other members of the cadherin family are also key players in gastrulation. Like *spadetail*, Snail is a transcription factor that is required for certain cells to move during gastrulation, but researchers have had few clues about the proteins Snail regulates. In the February issue of *Nature Cell Biology*, teams led by Angela Nieto at Instituto Cajal in Madrid, Spain, and Antonio Garcia de Herreros at Universitat Pompeu Fabra in Barcelona reported that Snail temporarily turns off the *E-cadherin* gene, which codes for a protein that helps epithelial tissues such as skin hold together. The result supports the idea that cadherins stop cells from migrating as they do during gastrulation, says Gumbiner.

That work may have applications to life beyond gastrulation as well: Many tumor cells, especially those with an ability to travel to new parts of the body, lack normal levels of E-cadherin. The researchers hope that drugs that block Snail might make tumor cells more sticky and less likely to spread.

As they try to link networks of signaling proteins to the molecules that trigger movement, scientists are getting help from new imaging techniques. For example, a team led by Kimmel and Richard Adams of Oxford University has developed computer-generated, time-lapse movies that trace the paths of individual zebrafish cells during gastrulation, which can pinpoint the motions that go awry in mutant embryos. In embryos with the *no-tail* mutation—which fail to develop a notochord or tail—cells begin to move at the normal time but seem to lose their way and do not gather at the midline, apparently because they lack a key molecule that controls the cells' compasses.

Such technologies, combined with new insights from molecular biology, bode well for solving the long-standing puzzle of gastrulation, says Kimmel. "Technically we are able to do so much more than a few years ago," he says. "It's a fantastic world ahead. We're right on the edge of some wonderful stuff."

–GRETCHEN VOGEL