A Puzzle: How Similar Signals Yield Different Effects

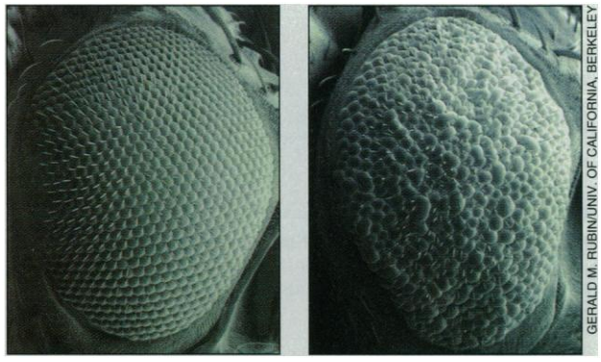
Life is a social enterprise. And one of its hallmarks is peer pressure: We become what we are to fit in with those around us. Now developmental biologists are beginning to find that even embryonic development is a social undertaking, in which constant chatter between cells in developing tissues determines their fates. To developmental geneticist Gerald Rubin of the Howard Hughes Medical Institute and the University of California, Berkeley, these cellular conversations are reminiscent of kids on a playground saying things like: "I'm it, so you can't be it" or "I'm it, so you have to be it, too." Says Rubin, who studies eye development in the fruit fly: "Peer pressure from neighboring cells is clearly the way most of development works."

To determine precisely how peer pressure nudges a cell toward one fate instead of another, many developmental biologists are now focusing on the medium of communication: the pathways by which cells detect and act upon signals from their neighbors. And one of the most intriguing findings from these studies is that many signaling molecules and pathways are reused time and again, turning up in species from fruit flies and worms to mice. What is more, biologists are finding that the same pathway may regulate the development of wildly diverse cell types.

And that presents developmental specialists with a major puzzle: How can similar signaling pathways shape so many different developmental processes? The rewards for solving that puzzle are great, because if researchers can solve it, they will be well on the way to uncovering the solution to the age-old mystery of how cell diversity is generated during embryonic development. Says Rubin, "There are only so many outcomes and so many signaling molecules, and knowing how they're used in combination with one another is an absolutely essential part of understanding development."

An illustration of what Rubin is talking about is offered by the Ras pathway, one of the best understood of the "signal transduction" paths leading from the cell's outer membrane to the genes in its nucleus. This pathway, named after a key signaling molecule within it, the protein product of the cancer-causing *ras* oncogene, is a major pathway regulating growth in mammalian cells. In the late 1980s, when developmental biologists began identifying the genes that control cell fates in two of their favorite experimental species, the fruit fly *Drosophila melanogaster* and the roundworm *Caenorhabditis elegans*, they discovered that the proteins encoded by these genes are the fruit fly and worm equivalents of proteins in the Ras growth-control pathway.

For example, Rubin's group made one such connection in 1987 when the researchers found that the *sevenless* gene, which is needed for the development of a specific neuron, R7, in the fruit fly eye, carries the genetic code for a tyrosine kinase receptor.



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***Ras* path misstep.** Development of the right fruit fly eye went awry because of the abnormal *ras* gene expression.

This is one of the membrane-bound proteins that kicks off signal transduction through the Ras pathway in response to growth factors by adding phosphates to other proteins inside the cell. "It is most gratifying to see this emerging theme of biological universality," says developmental geneticist Robert Horvitz of the Howard Hughes Medical Institute (HHMI) and the Massachusetts Institute of Technology in Cambridge, whose group has found some of these connections between development and the Ras path in *C. elegans*. "That the process involved in the development of the fly eye can tell you something about oncogenesis is absolutely remarkable."

As they traced the Ras-mediated pathways, researchers learned that they are widespread among species and highly versatile. The pathway that guides eye development in *Drosophila*, for example, is nearly identical to one that directs vulval development in *C. elegans*. Moreover, even within the developing tissues of a single organism, the Ras pathway can be used to tell cells to do different things. During worm vulval development, a Ras-mediated signal may tell a cell to divide, not to divide, to become a neuron or to resist becoming a neuron, says Paul Sternberg, a developmental geneticist at HHMI and the California Institute of Technology in Pasadena.

Because the pathways themselves are so similar—but the outcomes are so varied—researchers immediately began to wonder what makes the difference in the responses. In some cases, argues Sternberg, the strength of the incoming signal may be important in determining how a cell will respond. This idea was originally suggested in 1986, when Sternberg and Horvitz showed that the fate of a worm vulval cell depends upon its distance from the signaling cell, and therefore presumably on the intensity of the signal that activates the Ras pathway, although that hasn't yet been proved directly.

But just as not all teenagers start smoking in response to peer pressure because they come from different homes, it is clear that a cell's response to Ras pathway signals depends partly on "the previous history of the cell," says Sternberg. A crucial part of that history is the impact of previous communications. Those earlier signals have elicited changes in the target cell, some of which undoubtedly occur at the end of signaling transduction pathways in the nucleus. There's evidence that during development the structure of the chromatin, the complex of DNA and proteins that forms the chromosomes, changes so that in some cells certain genes may be primed to respond, while in other cells these same genes may be inaccessible to the transcription factors that control their activity.

Exactly what those genes might be is still one of the big mysteries of development, however. "It's another whole question, which genes have their transcriptional activity regulated by a given signal transduction pathway," says Jim Posakony, a developmental geneticist at UC San Diego. "We must know that if we want to know how developmental signals actually change the properties of cells."

A cell's previous history can change the way it responds to signal transduction without necessarily altering gene activity, however. For instance, researchers find that cell-surface receptors may be moved around or engulfed into the interior after they bind a signal-transmitting molecule. Or the activity of receptors or other pathway components may be modified by existing enzymes. Take the development of the fruit fly sensory bristle. This exterior organ, which consists of one neuron and three different accessory cells, is derived from ectoderm, one of the three primordial germ layers that give rise to all the body's tissues. Sensory bristle development is regulated by the Notch pathway, an apparently ancient and widespread signaling pathway that comes into play at many stages during development of the fruit fly's peripheral nervous system.

Studies have shown that Notch-mediated signals inhibit the capacity of ectodermal cells to become neurons, but they don't affect all ectodermal cells the same way. "If we take away the activity of one gene in the Notch pathway, instead of getting one neuron in the sensory bristle, we get four neurons," says Posakony, who is studying the pathway. That is, the Notch-mediated signal prohibits three of the cells from becoming neurons, but not the fourth. Exactly why the cells respond differently is unclear, but Yuh Nung Jan of UC San Francisco has evidence that it might be because some cells have previously acquired more of a membrane-associated protein called Numb than others. Numb may be a negative regulator of the Notch pathway, Posakony says, in which case those cells that have greater amounts of the protein may fail to respond to Notch signals and go on to become neurons, while the others become accessory cells.

Although researchers have some clues to how similar signaling pathways can cause different responses, no one expects that getting the full picture will be easy. The problem is complicated by the fact that cells are rarely exposed to one signal at a time, but are usually under a barrage of signals, some positive, some negative. "The real challenge will be to find out how a cell getting signals from, say, five different receptors integrates them and makes the right decision," says Rubin.

While that may be difficult, fully understanding development will require that researchers accomplish the arduous task of fitting the various signal transduction pathways into a complex model showing their overlapping relationships with each other and with additional regulatory molecules. Says Igor Dawid, a developmental biologist at the National Institute of Child Health and Human Development in Bethesda, Maryland: "There's no way around knowing all the players and their interactions. Eventually some people may throw up their hands and say it's too complicated, but you have to know who talks to whom and how it works, or else you don't understand it." Indeed, the cross-talk and intense peer pressure directed at a cell during development could easily rival the loud taunts, competition, and coercion seen on a busy playground.

–Karen Schmidt

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Additional Reading

H. Maruta and A. W. Burgess, "Regulation of the Ras signalling network," *BioEssays* **16**, 489 (1994).

W. S. Katz and P. W. Sternberg, "A plethora of intercellular signals during *Caenorhabditis elegans* development," *Current Opinion in Cell Biology* **4**, 939 (1992).

MEDICAL APPLICATIONS

Moving Developmental Research Into the Clinic

George Bernard Shaw thought youth was wasted on the young. In the same way, some clinicians think embryogenesis is wasted on the embryo. Starting from a few unspecialized cells, the embryo can spawn all the new cells and tissues needed to provide an organism with its correct complement of organs. Unfortunately, the adult doesn't possess that full range of biological skills. But if similar capacities could be bestowed at will on the adult human, many diseases could be cured simply by growing new tissues to replace damaged ones. "A very important medical goal," says developmental biologist Randal Johnston of the University of Calgary, "is to be able to emulate embryonic conditions and trigger cell proliferation in adults where these abilities are diminished."

With that goal in mind, industrial and academic developmental and cell biologists are exploring a variety of approaches to harnessing the embryo's generative powers to provide new therapies for disorders as diverse as diabetes, blindness, heart failure, neurodegenerative diseases such as Alzheimer's and Parkinson's, hard-to-heal wounds, and broken bones. Some researchers, for example, are testing the prospect of transplanting embryonic cells into the adult as a means of regenerating worn-out tissue. But that approach suffers from more than the practical obstacles that plague any new therapeutic strategy; it has the drawback that any use of fetal tissue is ethically controversial.

The approach that's the most advanced clinically doesn't suffer from that problem, however. It takes advantage of the fact that many of the protein "factors" known to trigger growth and cell differentiation during organ formation in the embryo do not become obsolete in the adult. In the embryo, these proteins impinge on every aspect of the developmental process, from the first laying down of the embryo's head-to-tail and dorsal-ventral axes to the formation of specific organs like the kidney, brain, and bone (see Article by Kessler and Melton on p. 596). And while their organ-building capabilities may not be so versatile later in life, they do help maintain and repair tissues and regulate their responses to the external environment.

Some of these proteins are already in clinical use: for example, erythropoietin, which triggers red blood cell production from stem cells in the embryo's yolk sac, liver, and spleen and in the adult's bone marrow, is used to treat the anemia that can be caused by kidney disease and by cancer and AIDS therapies. Over the next few years, clinical applications of growth and differentiation factors can be expected to explode as more are unearthed and more uses are found for those already discovered.

One of the new "embryonic" therapies that is closest to clinical application uses the proteins to restore damaged tissues. This therapeutic strategy is based on "a very nice rationale," says the National Cancer Institute's (NCI's) Michael Sporn, namely, that "the repair of soft and hard tissue in the adult is nothing but a recapitulation of what goes on in the embryo."

As an example, Sporn cites a protein called transforming growth factor β (TGF-β), which he and NCI colleague Anita Roberts helped identify back in 1983. TGF-β was named because in a culture medium it causes some cells, such as the fibroblasts that form connective tissue, to divide uncontrollably, much as if they have undergone a cancerous transformation. But TGF-β and certain related proteins also play a role in normal de-

SOME GROWTH OR DIFFERENTIATION FACTORS IN CLINICAL TESTING

Factor	Application	Status
Erythropoietin	Anemia due to kidney failure	FDA approved
Nerve growth factor	Peripheral neuropathies	Early trials
Insulin-like growth factor	Diabetes	Midphase trials
Brain-derived neurotrophic factor	Lou Gehrig's disease	Early trials
Transforming growth factor β	Macular holes (retinal wounds)	Late trials
Platelet-derived growth factor	Diabetic skin ulcers	Late trials
Osteogenic protein	Bone healing	Midphase trials
Granulocyte colony stimulating factor	Stimulating white blood cells after marrow transplant	FDA approved