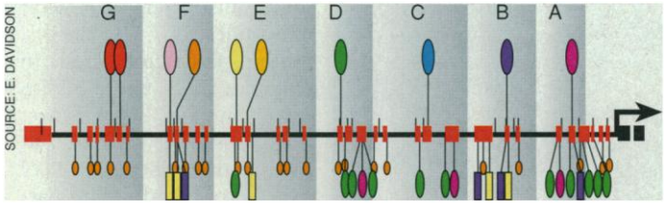
"Smart" Genes Use Many Cues to Set Cell Fate

The ball of undifferentiated cells that makes up an early embryo, some biologists have suggested, is like an orchestra without a leader. Each musician—each cell—has a wide choice of parts to play. But egg and sperm have provided their unspecialized progeny with little direction about making this choice: Other than chemical or structural variations within the egg itself, there is scant notation telling one descendant cell to play a neuron, for instance, or another to become a muscle cell. Instead, researchers have speculated, most cells might activate the proper combination of genes allowing them to choose the right part at the right time by listening to the biochemical music of their neighbors—secreted regulatory factors such as hormones—for cues. Unfortunately, there hasn't been much evidence that the genes in these cells are "smart" enough to pick up the multitude of nuanced biochemical notes surrounding them.

Now there is. In the April issue of *Development*, California Institute of Technology (Caltech) developmental biologist Eric Davidson and colleague Chiou-Hwa Yuh report evidence of a sophisticated biochemical ear in a gene in the sea urchin *Strongylocentrotus purpuratus*. The gene is expressed in cells that become the gut of the urchin embryo, and it contains over 30 binding sites for at least 13 different transcription, or regulatory, factors that the cell generally produces in response to external signals. The sites act as the gene's "on-off switches" and "volume controls," and the researchers have discovered that they are clustered in DNA fragments or "modules." Each module responds to a different suite of transcription factors, and various combinations of modules can be put together to change the time and place of the gene's activation, and hence the cell's fate.



Command modules. Expression of the sea urchin gene *Endo16* in a developing embryo depends on a variety of transcription factors (large and medium colored ovals and rectangles). Various combinations bind at sites (red rectangles) divided among modules A through G on the gene's regulatory region. Proteins that help the DNA loop (small orange ovals) bind nearby.

Previously biologists had observed genes binding only a handful of transcription factors, so this study goes a long way toward showing just how many environmental cues genes are capable of picking up and integrating, other researchers say. "It's really a beautiful example of the combinatorial complexity of gene regulation," says Eric Olson, a developmental biologist who studies mammalian muscle cell specialization at the University of Texas Southwestern Medical Center in Dallas. Michael Levine, a developmental geneticist at the University of California, San Diego, adds that "Davidson has had an enormous impact on my thinking. ... The module may indeed be the fundamental unit of pattern"—the creation of structure and difference—"in the early embryo."

Davidson's own thinking on modular gene regulation stretches back to the early 1970s, when he and Caltech biologist Roy Britten first came up with the idea that a gene could follow the embryonic music without a conductor if its "*cis*-regulatory" region—a section of DNA, preceding the protein-encoding sequences, already known to set the volume of transcription—could accommodate many interacting transcription factors. The region, Davidson has since proposed, could act like a miniature computer circuit, using the presence or absence of various transcription factors as "data bits" in calculations of the gene's proper expression level. Until recently, however, there was little evidence for this kind of complex regulatory mechanism, says Davidson. "Most genes have been studied from the other direction"—starting with known transcription factors and determining their binding sites and individual functions, or deleting random chunks of DNA from regulatory regions—"and the potential complexity was underestimated, because people weren't looking for it."

But in the early 1990s Davidson and others developed the molecular and embryological techniques needed to examine the *cis*-regulatory circuitry as a whole. Davidson believed that a sea urchin gene called *Endo16*, which was discovered by Tufts University developmental biologist Susan Ernst and coworkers in 1989, might be a good target for such an examination. Every cell in the embryo has the gene, but its product, a surface protein thought to be involved in cell adhesion, accumulates only in the "vegetal plate" of the early sea urchin embryo, the patch of cells that gives rise to endodermal tissues such as the gut. Identifying the different parts of *Endo16*'s *cis*-regulatory region that respond to different signals, Davidson thought, might help show how it does this.

In 1994, Davidson, Yuh, and other Caltech researchers tested the binding of extracts containing thousands of sea urchin nuclear proteins to *Endo16*'s *cis*-regulatory domain, reasoning that areas of strong protein-DNA interaction were likely to be transcription factor binding sites. But when the search turned up a surprising number of such sites—nearly three dozen, grouped into seven clusters—some observers doubted that they all had functional significance. "Most people thought that nine-tenths of the sites would probably be functionally meaningless," says Davidson.

Yuh and Davidson dispelled the doubts by using restriction enzymes to separate the seven clusters, or modules, then attaching them in various combinations to a bacterial gene that produces an easily detectable "reporter" enzyme when it is active. The pair then inserted these artificial gene constructs into the genomes of sea urchin eggs. The reporter gene's activity was high in some areas of the developing embryos and nonexistent in others, depending on which construct had been inserted. This indicated that the combination of transcription factors present in each area of the developing embryo was different, and that these combinations were activating some of the modules.

The modules' additive effects seemed to determine where and when the gene was expressed in the embryo. Embryos containing constructs driven only by modules A or B, for example, expressed the reporter enzyme not just in the vegetal plate but also in adjacent areas usually fated to become skeletal and ectodermal cells. Adding modules E or F to constructs containing A or B, however, prevented enzyme expression in the future ectodermal cells, and adding C and D prevented it in the future skeletal cells as well. Modules A and B, therefore, seem to be designed to promote *Endo16* expression in response to transcription factors available in all three areas, while C, D, E, and F respond to more localized signals by blocking the function of A and B in cells where *Endo16*'s protein product isn't needed. Although Davidson had expected the system to be intricate, he says "I was amazed at the actual molecular complexity that our experiments revealed."

Complexity in even finer detail was revealed by Davidson lab member Carmen Kirchhammer's examination of another tissue-specific sea urchin gene, *CyIIIa*. This gene has three *cis*-regulatory modules and 20 binding sites that "listen" for nine different tran-

scription factors. Using the same reporter system, Kirchhammer showed that mutating or deleting individual binding sites changed the overall function of the modules, evidence that every one of the transcription factors performs a specific regulatory function. Certain sites within each module seemed to serve as key "on" switches, as mutating them disabled the entire module; other sites appeared to amplify the activity produced by the module, or to restrict it to certain regions of the embryo.

These interactions, Olson says, "are likely to provide a general mechanism for understanding cell-specific transcription during development." Moreover, Davidson's findings may also have "important evolutionary implications," says Levine. Because each *cis*-regulatory module seems to work on its own, transposing them from one gene to the next may be a convenient way for nature to generate novel patterns of development. And for biologists, it may generate an understanding of how the solo begun by a fertilized egg swells into a multicellular symphony.

–Wade Roush

Additional Reading

C. V. Kirchhammer and E. H. Davidson, "Spatial and temporal information processing in the sea urchin embryo: Modular and intramodular organization of the *CyIIIa* gene cis-regulatory system," *Development* **122**, 333 (1996).

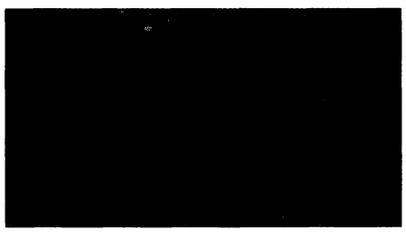
C.-H. Yuh and E. H. Davidson, "Modular *cis*-regulatory organization of *Endo16*, a gut-specific gene of the sea urchin embryo," *Development* **122**, 1069 (1996).

E. H. Davidson, "How embryos work: A comparative view of diverse modes of cell specification," *Development* **108**, 365 (1990).

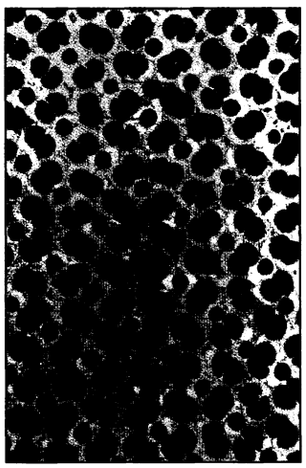
MACHINE VISION

Sunfish Shows the Way Through the Fog

Anyone who has driven in heavy fog knows how difficult it is to see things clearly in a medium that scatters light. Yet fish appear to move with ease through a dense aquatic fog of swirling algae, plankton, sand, dirt, and all sorts of plant and animal debris. Now, after studying the retina of the green sunfish (*Lepomis cyanellus*), researchers at the University of Pennsylvania, Philadelphia, think they know how this creature, at least, accomplishes the feat. And they are trying to copy the sunfish's presumed strategy, known as polarization difference imaging, to improve the vision of cameras in murky conditions.



MICKEY P. ROWE



STEVEN S. EASTER

Double vision. The green sunfish *(above)* has a pattern of double cones in its retina *(right)* that may produce two orthogonally polarized images.

"The really interesting thing is that it has a real application, for example, landing airplanes in fog," says physiologist James Larimer of the NASA Ames Research Center. "Now that we begin to understand how it works, we can actually build sensors that behave just like these animal systems do and extend the range by a substantial amount."

The retina of a sunfish, like that of humans, is a forest of guides focusing incoming light onto detectors. But Mickey Rowe of the university's Institute of Neurological Sciences and his colleagues discovered that there is a crucial difference between humans and sunfish, as well as some other animals, in one type of retinal light guide, known as "cones": The sunfish's cones come in pairs. Each pair transmits light as a single light guide with an elliptical cross section.

Rowe and his colleagues believe that the cone pairs guide light with different polarizations preferentially: Incoming light vibrating parallel to the long axis of the ellipse is transmitted more efficiently than light vibrating across the ellipse. They also found that the cone pairs are arranged in an array, with half the pairs aligned in one direction and the rest aligned in a perpendicular direction. The sunfish therefore seems to be seeing two orthogonal polarized images of the world.

Rowe believes the fish uses the two images to do some clever image processing. When light is reflected off a solid surface, it often becomes partially polarized. Rowe believes the sunfish can filter out light scattered from particles in the water, which has no particular polarization, by subtracting one polarized image from the other. What remains is light that is strongly polarized, and therefore most likely to have traveled, undeviated, from the solid object to the eye.

In an issue of *Applied Optics* last month, Rowe's team, along with Scott Tyo of the university's School of Electrical Engineering, showed that this subtraction technique can be used in a camera system to improve the visibility of some objects in a scattering medium by a factor of 2 to 3. In a bath of milk and water, the team illuminated a circular metal disc with two square patches etched on it that partially polarize light. They took two pictures through the murky water—first with a polarizing filter in one direction in front of the camera and then in a perpendicular direction.

When the researchers subtracted one of the two pictures from the other, the abraded patches consistently showed up more clearly than when they added the images together, which simulates what a normal camera would see. The team saw improvement even when the milk was so concentrated that less than 1% of the light reaching the camera was polarized. "What these guys have is an elegant way of processing polarization information," says David Williams, professor of brain and cognitive sciences at the University of Rochester, New York. This kind of "opponent processing" system is ubiquitous in nature, he says.

There are some problems, however, that the researchers will have to overcome before the system can be put to practical use. For a start, the technique only works if the polarizing filter on the camera is "in line" with the polarization of the reflecting object; otherwise the technique can make visibility even worse. To get around this, Tyo designed a system that automatically rotates the filters to optimize the polarization axes of the camera. Medical imaging specialist Robert Alfano at The City College, City University of New York, also points out that not every reflecting object polarizes light: "If the objects have a preferential axis of reflecting [polarized] light, they can use this method to see it. If not, they can't."

Tyo is aiming to tackle this question, but he points out that many of the objects a machine vision system might want to see—such as a road or the surface of the sea—do polarize strongly. He adds that dielectric materials—which include such things as fish scales—inherently polarize light well. So there is one natural object that polarization difference imaging can definitely see: the green sunfish.

–Sunny Bains

Sunny Bains is a technology writer based in Edinburgh, U.K.