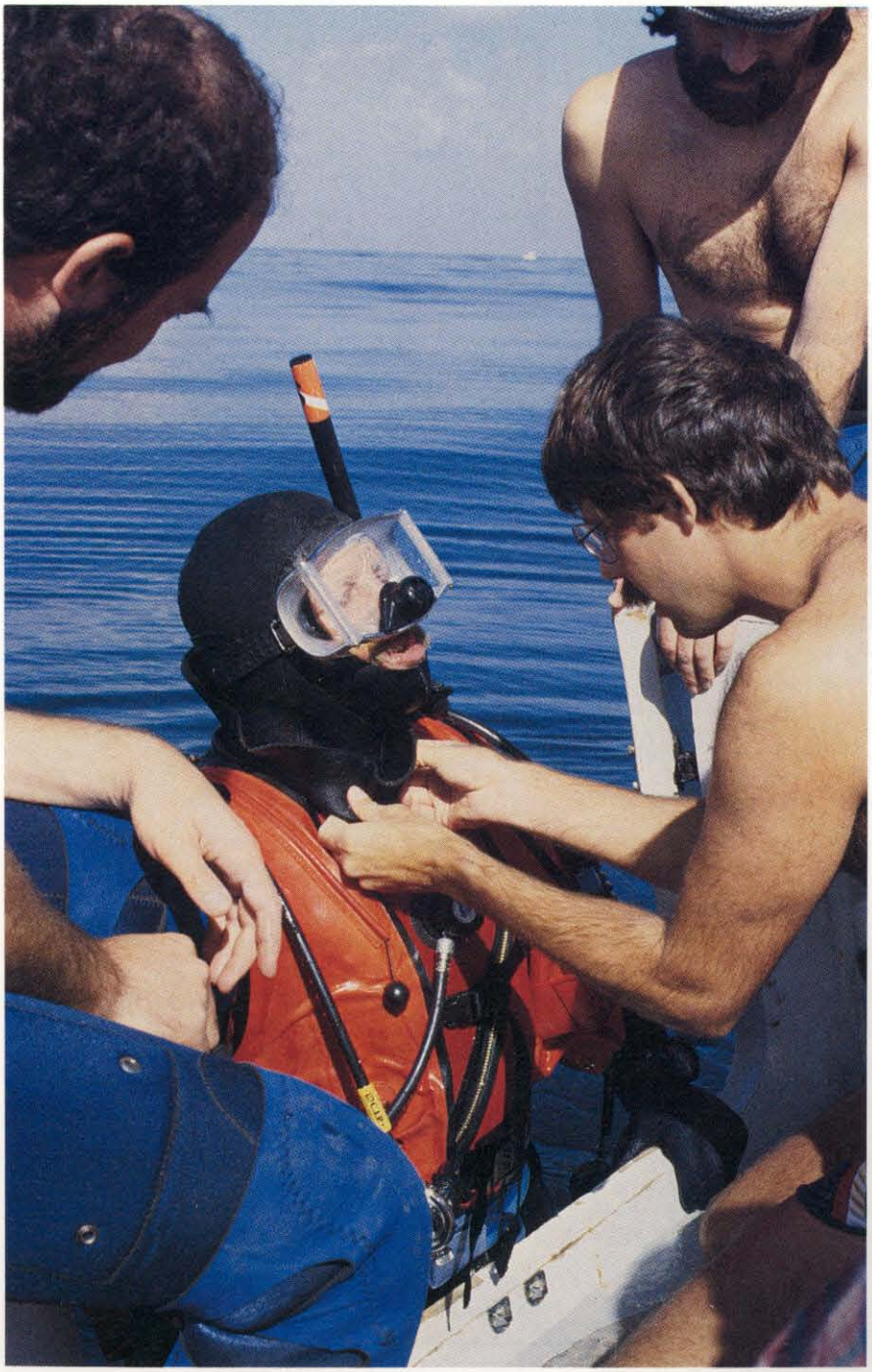


Above: Eric Davidson (in diving gear) prepares to dive off Point Loma for sea urchins that live under rocks 60 ft. down. Only these deep-dwelling, non-seasonal sea urchins spawn year round, providing a steady supply of eggs for Davidson's laboratory. Other divers on this expedition last fall included grad student Henry Sucov (left), postdoc Steve Fain (top right), and Pat Leahy (right), technician at Kerckhoff Marine Laboratory.

Top: A fluorescent dye injected into one cell of the embryo at its eight-cell stage lights up all the daughter cells derived from that cell in this mature sea urchin larva containing 1,500 cells. The face of the larva is toward the top, and the stomach is obscured by the fluorescent cells. (Photo by R. Andrew Cameron)

Bottom: A sea urchin releases thousands of eggs into a beaker.

From Cell to Organism:

Discovering the Mechanisms of Development

THE COMMON CALIFORNIA purple sea urchin, *Strongylocentrotus purpuratus*, although a pest to kelp harvesters, is a valuable commodity to developmental biologists. In just 72 hours a single fertilized sea urchin egg turns into a relatively complex organism of 1,500 cells — a larva that can swim, feed, and maintain itself. Sea urchins are plentiful, and they produce thousands of eggs at a time, which proceed to develop synchronously. What's more, the embryo is transparent and permeable, that is, you can introduce tracer molecules and watch what happens as development unfolds.

Over the past hundred years this favorite experimental subject has provided great insight into the processes of embryogenesis. Now, with the new techniques of recombinant DNA the lowly sea urchin is helping to elucidate the answer on a molecular level to the fundamental question of developmental biology: How does a complex organism with many kinds of cells develop out of a single cell?

"We want to know the nature of the process encoded in DNA that transforms genetic information into three-dimensional structure," says Eric Davidson, the Norman Chandler Professor of Cell Biology. Cells with different functions are determined by the differential expression of particular genes. But since each cell contains in its nucleus the entire genome, that is, all the genes possessed by that organism, the fate of any one cell is directed by a particular set of genes turning on at a particular time and place. How do the genes know when and where they are supposed to turn on?

Davidson and his group, including his long-time collaborator, Roy Britten, Distinguished Carnegie Senior Research Associate, study on a molecular level the very earliest of these processes in the sea urchin embryo. The unfertilized egg itself is not symmetrical. It consists of two distinct hemispheres that will give rise to distinct types of cells. So there are potential spatial cues already present in the original cytoplasm. As the egg begins to divide after fertilization, a crucial series of spatial reorganizations sets the stage for the differential expression of genes that will create the different cell lineages.

With radioactive probes as molecular markers Davidson and his lab have tracked differential gene expression in the cell lineages of the sea urchin embryo's gut, skeleton, and ectoderm (the layer of cells that will ultimately give rise to the larva's single-cell-thick body wall), trying to trace the mechanisms back to their beginnings, long before any visible change in embryonic structure. Differentiation in each of these lineages, they have found, begins at a different time and follows its own regulatory signals. The earliest evidence of gene differentiation they have discovered occurs in the ectodermal cells. At 10 to 12 hours after fertilization, a gene called CyIIIa first turns on. This gene codes for the protein actin, an essential ingredient of ectodermal cellular structure. What is interesting is that CyIIIa, although it exists in all the early cleaved cells of the embryo, turns on only in those cells whose offspring will give rise to the ectoderm.

To track this mechanism further, the researchers had to follow the actual functioning of the gene. "If you can put genes into an embryo and they work, you can find out what makes them work," says Davidson. He and his group fused the CyIIIa gene to a bacterial gene with an easily recognizable product, CAT (chloramphenicol acetyltransferase). This fused gene is injected into the unfertilized egg — anywhere in the egg. By observing when and where the CAT gene is activated, they have located the gene's regulatory sequences, which lie along the DNA strand immediately next to the gene they are regulating. These regulatory sequences apparently spring into action when they are recognized and bound by other molecules — proteins called trans-regulators, which have been looking around the cell nucleus for precisely those sequences. Since the researchers knew that the CyIIIa-CAT gene was not localized to a particular neighborhood, they can conclude that the trans-regulators must somehow be trapped in certain areas, producing a heretofore invisible asymmetry.

There still remains the question of the identity of these trans-regulators and how such proteins are distributed to produce this spatial organization in the early embryo. They might derive from maternal spatial assignments already present in the unfertilized egg. Or the trans-regulators might be localized by movements in the egg cytoplasm after fertilization. A third possibility is that contiguous cells could affect each other in ways that could cause synthesis or release of these molecules.

Although there will undoubtedly be some basic similarities, these mechanisms may turn out to be different for each stage of development in each organism — what's true for the sea urchin will not be true for the frog or the fruit fly or the human being. Since these very differences and similarities between systems can be so illuminating, it is important to study development in a wide variety of organisms. Multiple approaches can provide comparative insights from different angles into the basic question of how genes function.

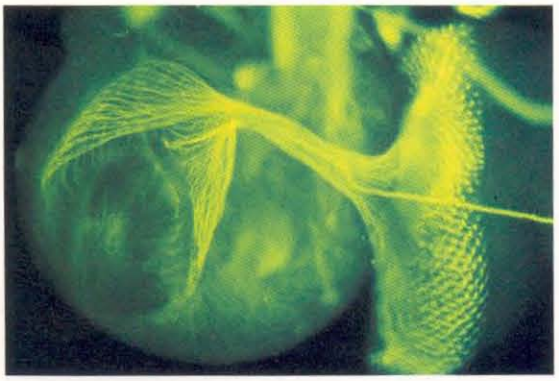
Flies' Eyes

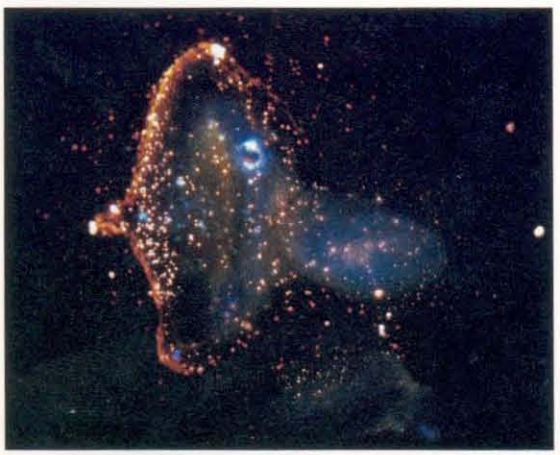
Seymour Benzer, the James Boswell Professor of Neuroscience, compares the different approaches of developmental biology to the old joke about the blind men feeling an elephant: "One is feeling the gene, one's feeling the cell, and another the embryo. But it's really the same huge problem we're all working on. We know a set of monkey genes makes a monkey; all we have to do is fill in the gap." Benzer is actually filling in the gap between fruit fly genes and fruit flies. Caltech possesses one of the world's most important repositories of mutants of *Drosophila melanogaster* — 1,500 different strains. The Institute has been a major center of *Drosophila* research ever since Thomas Hunt Morgan, the founder in 1928 and first chairman of the Division of Biology, began using *Drosophila* to explore some basic theories of genetics. The tiny fly with a convenient 10-day life cycle went on to become a favorite of geneticists everywhere. And as new techniques such as recombinant DNA shed new light on molecular processes, Caltech has become a place where classical genetics and molecular biology are closely intertwined.

Benzer and his associates approach a later stage of development than Davidson does, concentrating on the late larval stage when the adult eye is forming and its neurons making the appropriate connections. *Drosophila's* compound eye provides a highly regular repeated pattern — 800 identical modules, each containing eight photoreceptor neurons arranged in a pattern of six cells surrounding two others. These neurons make three different types of connections — the six surrounding cells form a particular synaptic hookup to the optic ganglia while the other two form different synapses at another level. Benzer's group has determined that these different cells do not descend in neat cell lineages from different parent cells. Rather, they all start off equivalent, and who's who among the eight is determined by interactions between cells based on positional information.

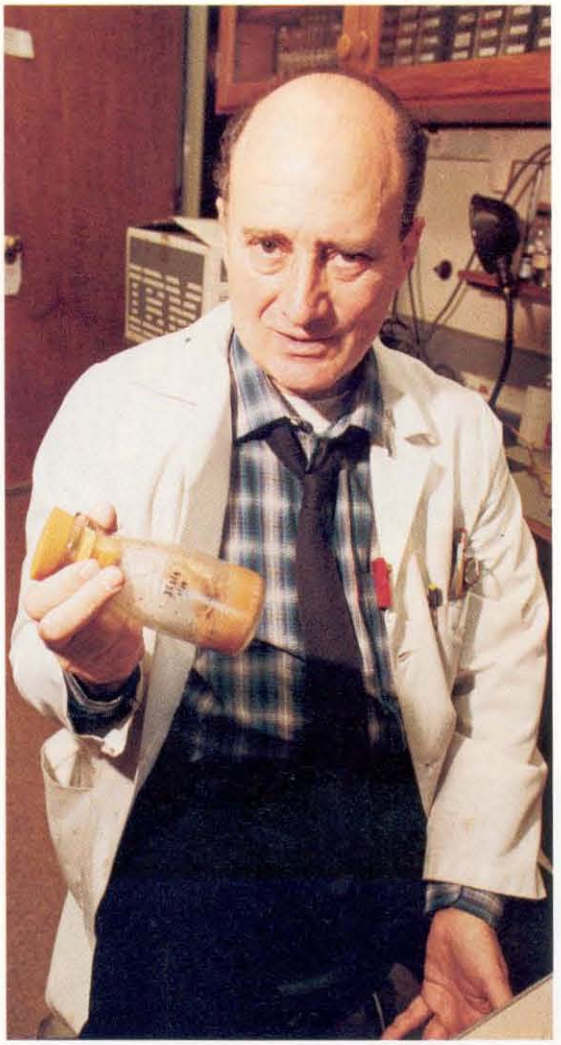
As the adult eye is forming in the mature larva, a wave called the morphogenetic furrow moves across the disk of the developing eye. In front of the wave the cells destined to become the photoreceptor neurons are all alike and are randomly arranged. After the wave has passed, they're grouped together in the six-surrounding-two clusters, with each of the three types sending its characteristic axons to the proper place on the optic ganglia.

Benzer wants to find the molecules associated with this wave that cause this cell differentiation. One of the new techniques of





Above: In this developing Drosophila *eye disk, the bright white dots at left signify the presence of the messenger RNA to an activated photoreceptor-specific gene. This photoreceptor gene turns on early in the eye's development and stays on until the larva emerges as an adult fly. (Photo by John Pollock)*

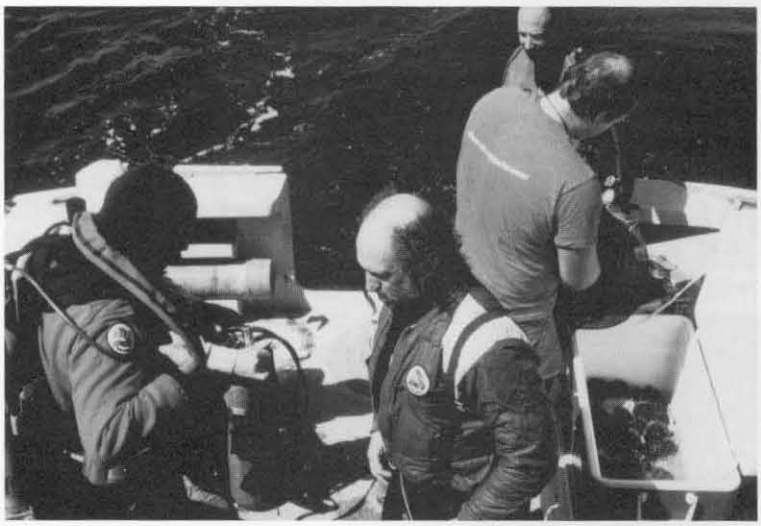


Top left: The developing Drosophila *adult eye (right) was stained with a monoclonal antibody (and fluorescent dye) specific to axons. The photoreceptor axon bundles can be seen entering the optic stalk (center) and fanning out inside the brain (left) toward the developing visual formation center. (Photo by Pat Renfranz)*

Left: Seymour Benzer displays a bottle of Drosophila *from Caltech's extensive collection of mutant flies. The flies live in old-fashioned milk bottles; the layer on the bottom is their food.*

Left: Elliot Meyerowitz inspects an Arabidopsis *plant (visible in its full glory below) in his basement-closet garden.*

Right: Roy Britten injects potassium chloride into a sea urchin recently hauled from the depths. This will make it release its eggs if it's gravid. The divers seek non-seasonal colonies of sea urchins that are not all gravid at the same time.



Below: Back on board, the divers (Davidson at center) relax while the sea urchins are packed in ice chests for the trip to their new home at Kerckhoff Marine Lab. This expedition netted about 600 animals to replenish the lab's population.

revealing such molecules is that of monoclonal antibodies, which recognize and bind certain antigens. When these are tagged with fluorescent labels, they light up on meeting their targets. Benzer's lab has developed a panel of 150 such monoclonal antibodies targeted at the *Drosophila's* nervous system. Although a number of them lit up at different stages of the development of the eye, one in particular, named MAb24B10, proved to be highly specific for the photoreceptors in a crucial stage.

The researchers could now take the antigen, or protein, recognized by MAb24B10, purify it, and determine its sequence of amino acids. This sequence was then compared to a similar sequence in the *Drosophila* DNA "library" to discover the gene that produced the protein. When genes are located on a particular chromosome, they can then be altered to produce mutations. Mutations of a defective gene will indicate the function of the original sought-after molecule.

"That's the great thing about *Drosophila*," says Benzer. "The process is a closed circle. You can enter at any point on the circle — at the gene, the antibody, or the behavior, wherever it's convenient — and determine the rest of the information."

Flower Genes

For this sort of circular process *Drosophila*, with its advantages of a long history of cataloged mutations and its adaptability to modern molecular techniques, makes a perfect experimental subject. But Elliot Meyerowitz, taking his cue from *Drosophila*, is on the trail of what may be an even better one. Only it's a plant.

Meyerowitz, associate professor of biology, still works with fruit flies, deciphering the regulatory sequences of genes that code for proteins produced in the larval salivary glands and secreted to form a glue that attaches the pupal stage of the insect to a surface. But his discovery of the beauty of *Arabidopsis thaliana* is leading him in other directions.

Beautiful in the ordinary sense it's not. "It will never replace the rose or the carnation in horticulture," says Meyerowitz. *Arabidopsis* is a member of the mustard family, a harmless weed with a tiny white flower and absolutely no commercial value. It's been used in classical genetic experiments for more than 40 years; its generation time of only five weeks and its production of 10,000 seeds per plant make it attractive for cross-breeding. And its small size and modest requirements for growth make it possible to grow tens of thousands of them in a small space. In fact, they're so small, says Meyerowitz, that "you can even grow them at Caltech" (which is not known for its spacious farmlands). Meyerowitz grows his plants in a basement closet. "My wife taught me how to grow plants," he says. "I now pay a lot more attention to what she does in the yard." (He would try growing *Arabidopsis* in the yard, but it's too hot.)

Like *Drosophila*, *Arabidopsis* is well suited

to experiments in classical genetics. The flower has a simple, regular, geometric form of four petals, four sepals (the green leaves that enclose the bud), six stamens, and two carpels. Mutations can turn petals into stamens or sepals, stamens into carpels, and produce all manner of easily recognizable interconverted organs. (This is also common in horticulture, Meyerowitz explains; a many-petaled rose is really a mutation or set of mutations that turns stamens into more petals than the flower's original five.) Meyerowitz is especially interested in this sort of pattern formation.

But the little mustard plant is also, it turns out, ideally suited to the techniques of molecular biology. Meyerowitz's laboratory discovered that *Arabidopsis* has a tiny genome; its total complement of DNA consists of only 70 million base pairs — far smaller than that of any other flowering plant. It also has very little repetitive DNA; most of its DNA is organized as extremely long blocks of unique sequences of nucleotides. This makes it much easier to map the location of a small DNA segment. Meyerowitz and his group have already localized 25 random pieces of *Arabidopsis* DNA and expect to have 50 by the end of this year. Before long Meyerowitz expects to find one of these random locations turning up close to a flower gene (flower mutations have been plotted on the same map). Then the researchers will have a flower gene that they can clone with recombinant DNA techniques. The cloned gene can then be modified and introduced back into the mutant plant to determine its function.

Meyerowitz's lab has already cloned several genes not involved in the flowering structure, including a gene that encodes the large seed-storage protein, one that encodes the plant's light-induced chlorophyll-binding protein, and another that codes for alcohol dehydrogenase (which may keep the plant from drowning by overwatering). Because *Arabidopsis* genes will cross-hybridize with the genes of other plants, this work has significance for plants of more economic value, whose vastly more complex systems are difficult to study.

But the fundamental question is still the same, in Meyerowitz's mustard plants as in Benzer's fruit flies and Davidson's sea urchins: What are the intrinsic rules that say to one *Arabidopsis* cell, "You're going to turn into a petal," or to a sea urchin cell, "You're going to become part of the gut"?

Bird Brains

Mark Konishi comes at the problem of cell differentiation from a different direction. Konishi, the Bing Professor of Behavioral Biology, is interested in the development of behavior — specifically birdsong.

Male zebra finches sing; females do not. Finch sons have to learn how to sing from finch fathers. But the capability for singing lies in an area of the brain that is developed in males and not in females. The search for the origins of this sexual dimorphism has led Konishi closer and closer to molecular biology for an answer.

The mature male zebra finch possesses more neurons of larger diameter in the song-control area of its brain than does the female. The sexes do not, however, start off that way. Up until 12 days after hatching, their brains look identical. Then, between 12 and 35 days of age, dramatic changes take place: In the male the nerve cells in the song-control area grow, while in the female they atrophy and die. Actually, all known cases of brain sexual dimorphism — in mice, rats, gerbils, and probably in humans, as well as finches — involve cell death in the female.

In experiments Konishi has found that the divergence between the sexes in the zebra finch can be modified by implanting estrogen under the skin of newly hatched female chicks. The song-control cells of those chicks do not die but grow some of the male characteristics. (It's not so strange that the female hormone, estrogen, should promote masculinization, says Konishi. Although testosterone, the male sex hormone, masculinizes a rodent brain, it works by being converted to estrogen by a brain enzyme.) The discovery that these nerve cells in the zebra finch die because they lack a particular chemical has wide-ranging implications far beyond sex differences. Certain human diseases, such as Alzheimer's and Parkinson's, result from nerve cell death. "So it's interesting to know what chemicals might maintain nerve cells," says Konishi.

The researchers don't yet know how the process works. Since females implanted with estrogen on the first day after hatching continue to develop masculine song characteristics long after the hormone's effect has expired, it may be that estrogen is only a trigger. It's also not yet known whether the natural neuronal growth of the *male* is actually due to the hormone. Other investigators have demonstrated an upsurge of estrogen in

Right: Mark Konishi holds two zebra finches seen in closer view below. The male, on the right, is distinguished by the golden patch on its face and the red beneath its wing. But more interesting to Konishi are the differences in the finches' brains.

The song-control areas, stained dark and indicated by arrows, are larger in the brain of the male zebra finch (right) than in that of the female (left).

the male chick's blood (and not in the female's) between two and five days after hatching. But this doesn't prove that the hormone is responsible for masculinization of the brain.

To try to observe these opposite processes of normal differentiation and cell death, Konishi and his group are growing finch brain cells in tissue culture. The first problem is to be able to determine which are the cells of the song-control area. They have recently developed a monoclonal antibody that labels almost exclusively the nerve cells of two of these areas, and they are encouraged that this antibody can be used to identify the song-control cells in tissue culture. Then the next step will be to see first *if* and then *how* and *where* estrogen works to make these cells thrive. "At least, if estrogen masculinizes tissue culture brain cells," says Konishi, "we have a strong hint that we can analyze the problem further." So far this has been a classical cell biology approach, but "we're now getting a bit molecular," he says. "It may lead to the level of how genes are expressed."

Konishi's lab contains large breeding colonies of zebra finches. Since it's important to know when they hatch, the eggs are collected and kept in an incubator. Zebra finches, an Australian species domesticated only recently, are "lousy parents," according to Konishi, who gives the chicks to Bengalese finches to raise. Since the more laid-back foster parents will adopt babies of any age at any time, the researchers don't have to be overly solicitous about their birds.

Developmental biology at Caltech, which crosses the interdisciplinary lines of neurobiology, cell biology, and molecular biology, includes many other researchers employing a wide variety of approaches and techniques to solving the question of how genetic information is translated into the patterns of life. Among them is Edward Lewis, the Thomas Hunt Morgan Professor of Biology, who has been working with mutant *Drosophila* strains since the 1940s, before anyone knew what DNA looked like (*E&S* September 1981). In creating an array of mutant flies with their body segments oddly hooked up and tracing the genes that caused these abnormalities, Lewis discovered a set of genes, called homeotic genes, that seemed to play the role of a "master control," switching other genes on and off. Assistant Professor Mark Tanouye also works with *Drosophila*, investigating the regulation of potassium permeability, which determines membrane excitability in nerve cells.

The classic laboratory mouse is also still alive and well at Caltech. James Bower, assistant professor of biology, uses mice to study how patterns of neuronal connections are established during the development of the central nervous system. Professor Elias Lazarides investigates how differential gene expression determines the structure of red blood cells (*E&S* March 1984). And Ellen Rothenberg, assistant professor of biology, studies the development of T-lymphocytes, which play a crucial role in the immune system (*E&S* March 1983, May 1984). Division chairman Leroy Hood, the Ethel Wilson Bowles and Robert Bowles Professor of Biology, has used embryo gene transfer techniques to cure "shiverer" mice of their genetic disease (see page 24).

Some of this work and that of still others at Caltech is being supported by a recent $12.5 million grant from the Lucille P. Markey Charitable Trust. Mrs. Markey, who died in 1982, owned Calumet Farm, a thoroughbred horse breeding and racing stable in Lexington, Kentucky. She had created the trust to support "the interdisciplinary efforts by small groups of able investigators who are addressing fundamental questions in biomedical science judged to be of potentially great importance." A symposium this month on "Cell Lineage and Specification in Development" (March 11-13) is marking the inception of the Lucille P. Markey Charitable Trust Program in Developmental Biology at Caltech. A number of internationally known scientists (several of them from Caltech) will present some of the latest advances in this field.

These advances have been made in a number of different organisms, each system providing another piece of the puzzle. All of the pieces will be necessary to see the whole picture of the diversity of life forms and how they arise. As Davidson says, "I believe that the means by which the correct spatial presentation of the necessary regulatory factors is arranged, the levels of hierarchy required, and the functions of the genes that are being regulated, will all turn out to be characteristic and distinct for each system of embryogenesis. It is in unraveling these very distinctions that we will meet, and solve, the real biological problem of explaining embryonic development in its many forms." □ — *JD*