Gone Fishing!

Scientists use mutant zebra fish to learn how vertebrate embryos develop

By JOHN TRAVIS

Mark C. Fishman has recently netted mutant fish so strange he has trouble believing they even exist.

Take the doomed animal whose blood cells literally fell to pieces when he exposed the fish to light. "The red blood cells became fluorescent and popped. You could watch the cells explode. It was really rather gorgeous," says Fishman, head of cardiovascular research at the Massachusetts General Hospital (MGH) in Boston.

The researcher didn't reel in this hapless mutant from the polluted waters of Boston's Charles River. Like scores of other bizarre fish, it was caught in Fishman's laboratory. The mutants are zebra fish, or *Danio rerio*, and they are the rage among developmental biologists, who study the seemingly miraculous process in which a fertilized egg cell becomes a multicellular adult organism.

Using radiation, chemicals, or viruses, these investigators deliberately trigger mutations in zebra fish in hopes of identifying the genetic repertoire employed by a developing vertebrate embryo. The fledgling field of zebra fish research hit a landmark this month with the publication of a special issue of DEVELOPMENT. In more than 3 dozen articles, researchers describe hundreds of mutant fish produced at MGH and at a laboratory in Tübingen, Germany.

"It's the largest collection of mutations affecting embryogenesis that exists for any vertebrate," says Wolfgang Driever, who organized the MGH mutant hunt and is now setting up his own zebra fish laboratory in Freiburg, Germany.

The journal celebrates its special issue with a bit of whimsy. The upper corners of the right-hand pages hold a series of 230 digitized images chronicling the development of a zebra fish embryo from its two-celled stage.

If you thumb quickly through the pages, "you can actually see cells dividing," says Donald A. Kane of the University of Oregon in Eugene, who worked on the flip book feature. "It's an incredibly fun time now to work with zebra fish. It must have been like this with flies many years ago."

Kane's remark about flies refers to the extraordinary success developmental biologists have had with *Drosophila melanogaster*, the common fruit fly. Last year, for example, a Nobel prize honored three researchers who in the 1970s studied mutant fruit flies in which embryogenesis has gone awry (SN: 10/14/95, p. 246).

While biologists have been startled to learn that many of the genes that guide insect embryogenesis perform similar tasks in vertebrates, there's only so much fruit flies can tell them. Consequently, scientists have longed for a vertebrate whose development is as easy to study.

The South African frog *Xenopus laevis*, whose embryo is quite large and easily manipulated, has been a leading contender. Yet two attributes make the frog a difficult organism in which to breed mutants and find the responsible genes. Instead of two sets of chromosomes, its cells harbor four. And in contrast to the quick-breeding fruit fly, the frog takes 2 years to reach reproductive maturity. As for the mouse, another popular laboratory animal, its embryos are hidden inside a mother's womb.

Perry Hackett

Normal Danio rerio*, or zebra fish.*

Enter the zebra fish. This colorfully striped freshwater fish, originally from streams in India, is often sold to people setting up their first aquariums. In the early 1970s, the University of Oregon's George Streisinger decided that zebra fish would make a great experimental subject for vertebrate biologists. Researchers now agree that Streisinger, who died in 1984 after more than a decade of research on the fish, made an inspired choice.

"This animal is very easy to take care of. They're extremely hardy, and they'll lay eggs like crazy under the right conditions," says Robert Ho of Princeton University.

It takes just 5 days for a fertilized zebra fish egg to complete embryogenesis. About 3 months later, that new fish can reproduce. "It doesn't take you very long to grow up a new generation," says Hazel Sive of the Whitehead Institute for Biomedical Research in Cambridge, Mass.

There's another major selling point to the zebra fish. "The embryos are extremely transparent, which is a boon to developmental biologists. We can watch even the deepest tissue interactions in the living animal," says Ho.

That ability particularly thrills developmental neuroscientists. "Between 10 and 24 hours [after fertilization] is when all the major subdivisions of the central nervous system become apparent," notes Steve Wilson of Kings College in London. "You can really see the nervous system go from a sheet of cells, the neural plate, to a fully structured brain."

After Streisinger's death, several scientists at Oregon carried on the zebra fish research tradition. For example, using irradiation and haploid fish, which have only a maternal set of chromosomes, the researchers produced a variety of mutants in which embryogenesis was disrupted. "The Oregon people haven't always gotten as much credit as they deserve," says Fishman.

Still, haploid fish don't develop properly. If zebra fish were to provide the best model of vertebrate development, researchers would have to screen for embryonic mutations in fish with a normal complement of chromosomes.

In the late 1980s, Christiane Nüsslein-Volhard, a researcher at the Max Planck Institute for Developmental Biology in Tübingen and one of the fruit fly scientists who won the 1995 Nobel prize, convinced colleagues that they should conduct a large-scale mutant hunt in an attempt to find the genes, an estimated 2,000 to 5,000, involved in normal zebra fish embryogenesis.

That ambitious effort, undertaken in parallel by the groups in Tübingen and Boston, has now paid off. Though the screens fell quite short of hitting every gene, researchers examined millions of embryos, ultimately identified mutations in about 600 specific genes, and have already described more than 300 of the most interesting mutants.

The two mutant screens differed slight-

ly in detail but followed the same general strategy. First, explains Fishman, "you take a male fish and dunk it in a chemical mutagen." This mutagen, a compound called ethylnitrosourea, randomly alters genes in sperm.

Through a laborious breeding process using those males and the offspring that followed, the scientists generated embryos whose development was lethally interrupted by a single gene mutation.

"It was an enormous amount of work," says Mary C. Mullins, a member of the Tübingen screening effort. In Germany, a building crammed with around 5,000 fish tanks was set aside for the project.

In the tradition of fruit fly researchers, zebra fish scientists have christened their mutants with quirky names that are only sometimes informative: *half-baked, avalanche, speed bump, zombie, ogre, lost-a-fin, piggy tail, snow white, bashful, sleepy, cyclops, mind bomb, uncle freddy, dog-eared, van gogh, silent heart,* and *throbless.*

The mutations seem to have tampered with almost every aspect of embryogenesis, including how an embryo knows its head from its tail and its abdomen from its back. Several mutations even disrupted epiboly, the very first cellular movements of the embryo. Unlike mammalian embryos, which derive nutrients from mothers via umbilical cords, zebra fish embryos depend on a yolk-laden cell until they can feed themselves. In normal epiboly, "cells spread over the yolk much like pulling a ski cap down over your head to your neck," says Kane.

Other mutant zebra fish had problems such as miswired nervous systems, pigmentation defects, eye abnormalities, tail malformations, or undeveloped muscles. "I was fascinated by the motility mutants," adds Mullins, who now works at the University of Pennsylvania in Philadelphia. "There were some mutants that would swim upside down and some that would swim in circles."

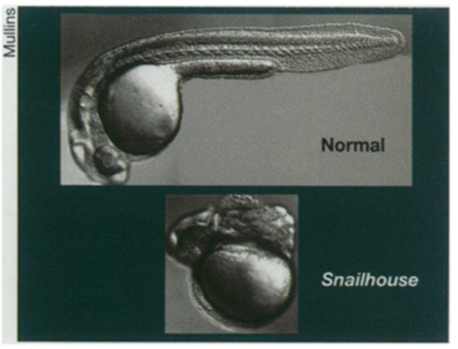
Among the more intriguing mutations are those that affect processes rather late in development and alter major organs, such as the gut, liver, pancreas, and heart. Scientists have had few chances to determine the genes necessary for such organ formation. "We have to go to a vertebrate to look at organ development because vertebrate organs are essentially different from those of *Drosophila*," says Fishman.

Fishman admits amazement at the variety of heart mutations uncovered. "I think they're breathtaking. For example, we'll have a mutation that selectively removes one chamber of the heart, some that put a chamber inside another chamber so that they're not oriented properly. You have mutations that affect just the formation of valves. Other mutations affect the thickness of the heart wall alone."

What the zebra fish mutants establish, he says, is that hearts are assembled by sets of genes that are responsible for discrete parts of the organ. "We didn't know such genes could exist," says Fishman.

Beyond defects in form, the screens found mutant zebra fish in which the heart did not maintain a proper rhythm. "The responsible genes may be very important clinically as well as to fundamental science. These mutants will hopefully give us a handle not only on congenital heart disease but propensity to heart disease," says Fishman.

Investigators have also identified zebra fish mutants in which blood formation goes awry. Remember the one whose blood cells explode? Appropriately named *dracula*, this mutant most likely has an enzyme deficiency that renders its blood pigment sensitive to light.



A mutant gene called snailhouse *disrupts normal zebra fish development (above), creating a malformed embryo (below).*

"It's a very curious mutation. It was originally thought this mutant developed no blood, but it turns out if you raise the fish in the dark, it does have blood," says Fishman. Related enzyme deficiencies, he adds, cause human diseases called porphyrias, one of which may have afflicted England's King George III.

While zebra fish have made a big splash in recent years, developmental biologists stress that the animals still have several flaws as research subjects. Scientists have yet to perfect the ability to add functioning genes to zebra fish, a talent that has proven extremely useful in mice. Nor can they deactivate a specific gene, another skill mouse researchers possess.

Moreover, zebra fish investigators have clearly trailed frog biologists in experimenting directly with the developing embryo and its cells.

"No one had done classical embryological assays in the zebra fish. No one had cut the embryos into pieces and sandwiched the little pieces of tissue together and asked what those sandwiches go on to become," says Sive.

"We know in frogs that to get the embryonic body plan set up, cells have conversations with one another. One group of cells signals, other cells respond, and the nature of those responding cells changes. The entire vertebrate body plan is set up by these cell interactions, or inductions. Yet we had no idea in fish where the inducing tissues were, where the responding tissues were, or what the sequence and timing of cell signaling events were."

In the June DEVELOPMENT, Sive and her colleagues began to answer those questions. In a series of experiments, they removed cells from the shield, a well-known part of the zebra fish embryo, and placed them next to cells from the animal cap, another embryonic region. The researchers observed that the shield tissue somehow signaled the animal cap to start developing into portions of the zebra fish nervous system.

"We could say for the first time that we had cells in the embryo that could induce neural tissue," says Sive. The exact nature of those signals, she adds, might emerge when researchers identify the genes responsible for the various mutants with disturbed nervous systems.

That remains a formidable challenge, however. "We have all these mutants, but we're still having trouble tracking down the genes," says Kane.

To find the single gene damaged in a mutant fish, most investigators have had to rely upon a laborious strategy called positional cloning. The first step involves looking for known genetic markers in the DNA of fish with and without the mutation. That enables scientists to establish that the mutant gene exists on a specific region of one chromosome. They must then identify every gene in that region and determine which is the altered one.

As researchers collect more and more of these DNA signposts—a new map of genetic markers appears in the DEVELOPMENT zebra fish issue—they'll home in on genes more quickly. Still, Nancy Hopkins of the Massachusetts Institute of Technology notes that it can take a lone researcher a year or more to positionally clone a gene.

In the Oct. 31 NATURE, she and her colleagues outline a different strategy for finding zebra fish genes. Instead of using chemicals to mutate genes, they have turned to viruses that integrate their genetic material into the fish's genes.

The viruses do not cause mutations as often as the chemicals do, but they do offer an important advantage. By scanning for viral DNA sequences, it's relatively simple to identify where a virus integrates its genes. This allows Hopkins' group to quickly find any mutated zebra fish genes. "We've already cloned three genes in just a few months," she says.

If her group can improve the frequency with which the viruses generate mutations, and if other researchers embrace the technique, Hopkins believes that finding the thousands of genes involved in zebra fish embryogenesis is realistic. "In 2 years, we could clone all the genes needed to make a vertebrate." □