Zebrafish Hit the Big Time

Vertebrate developmental biologists have hungered for a new animal model. Two decades of patient work on a small aquarium fish has given them one. Now the fun begins

For more than a decade, vertebrate developmental biologists have watched with fascination as the mysteries of early embryonic development were unraveled in the fruit fly *Drosophila*, right down to the molecular level. But the awe was mixed with envy, because the experiments that made this success possible couldn't be done in vertebrate systems. None of the established vertebrate models shares *Drosophila*'s virtues of breeding prolifically and producing readily observable embryos. Mouse embryos, for instance, develop deep within the mother's uterus, while another lab favorite, the African clawed frog *Xenopus*, breeds too slowly to be useful for genetic experiments.

As a result, detailed genetic analysis and sophisticated manipulations of vertebrate embryos were out of reach. And that's why, about 5 years ago, a small band of developmental biologists began pinning their hopes on the zebrafish, a small aquarium species that breeds quickly and produces large, transparent embryos that are a developmental biologist's dream (*Science*, 5 October 1990, p. 34). But to make the zebrafish a useful model, researchers had to find a wide range of mutations that disrupt embryonic development and devise the means to isolate the mutant genes responsible for these effects.

Now the first of these goals has been achieved, following an exhaustive screen of almost 2 million embryos by the groups of Christiane Nüsslein-Volhard at the Max Planck Institute for Developmental Biology in Tübingen, Germany, and Wolfgang Driever at Boston's Massachusetts General Hospital. Many developmental biologists, who are just learning the details of the teams' harvest of 2000 developmental mutants, have been left groping for superlatives. "The screens are a spectacular success," says frog-biologist-turned-fish biologist Tom Sargent of the National Institute of Child Health and Human Development (NICHHD) in Bethesda, Maryland. "[They] will make a very big difference...to the way all vertebrate development is studied." And with other labs making rapid progress in producing genome maps that will help in isolating zebrafish, he predicts, "the field is really going to take off."

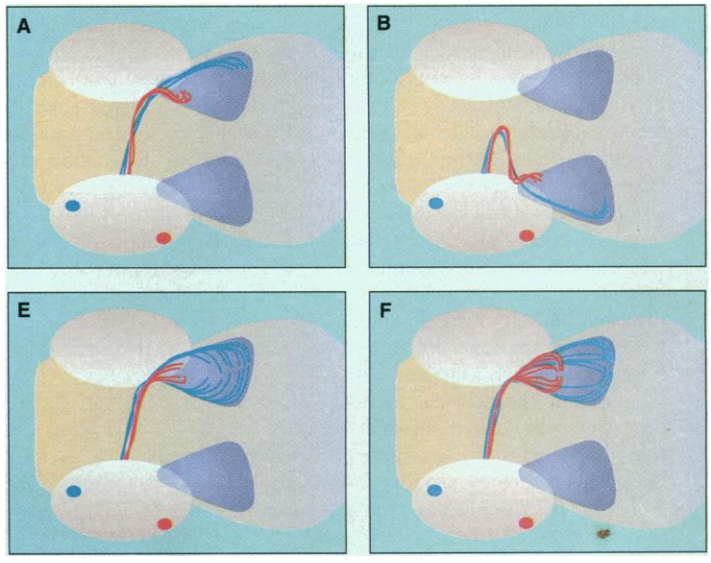
The key to the zebrafish mutant search is a strategy called "saturation mutagenesis" pioneered in 1980 by Nüsslein-Volhard and Eric Wieschaus, then both at the European Molecular Biology Laboratory in Heidelberg, Germany. That approach produced the *Drosophila* mutants that propelled the fruit fly to the center stage of developmental biology. The basic idea is simple: Treat adult males with a chemical mutagen and search, three generations later, for embryos that develop abnormally. Because developmental pathways are controlled by cascades of molecular events, however, getting a complete picture of development depends on hitting all, or nearly all, of the genes influencing each cascade. And this, in turn, means screening vast numbers of animals, the precise number depending on the efficiency of mutagenesis.

Nüsslein-Volhard calculated that she would need to screen more than 1 million zebrafish to match her previous success in *Drosophila*. And when she set up the first zebrafish tank on her office windowsill in 1987, that seemed an impossible undertaking, since no one had ever raised zebrafish on such a massive scale. Indeed, this obscure fish had been studied in detail at only one research center: the University of Oregon at Eugene, where the late George Streisinger, Chuck Kimmel, and others began exploring the potential of the zebrafish as a developmental model in the early 1970s.

It took Nüsslein-Volhard's group 5 years to refine the techniques needed to run a fish colony large enough to do the job. The key, they found, is squeaky clean water, and so they designed their own aquarium system to provide pure water while minimizing space, cost, and maintenance time. Diet was also a problem, since the freshly hatched fry require live food. But eventually the researchers perfected a mix of live paramecia and freshwater invertebrates, while discovering that adults flourish on a more readily available food—fruit fly larvae. And while most top researchers would probably balk at having to spend years perfecting techniques for raising fish, Nüsslein-Volhard says it meant the difference between success and failure: "This is all absolutely crucial. Many people haven't grasped this."

By fall 1992, a new facility able to accommodate 350,000 fish was ready. The following spring, 12 members of Nüsslein-Volhard's group began the screen. After the exhaustive groundwork, finding signs of abnormal development was fairly easy, because it's possible to see deep within the transparent embryos.

More than 1 year and 1.2 million embryos later, Nüsslein-Volhard's screen has yielded some 1300 mutants that produce a dazzling array of abnormal phenotypes. Some show defects in the cell movements that occur during gastrulation, the process that yields the first distinct embryonic cell layers; other embryos have an abnormal body plan. Most, however, have defects that show up only later on in development, including some that are abnormal in just one structure (notochord, brain, or jaw arches, for example); in others the abnormality affects a particular tissue type (too little muscle, say). Says Scott Fraser, a zebrafish biologist at the California Institute of Technology in Pasadena: "Instead of everyone in the field talking about

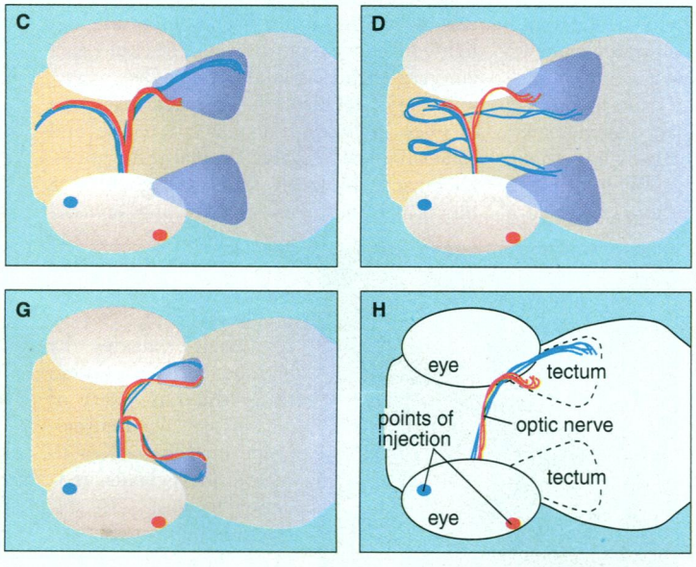


Going astray. Diagrams of zebrafish mutants with defects in connections between retina and tectum (a brain structure involved in vision). A and H show the normal pattern; B through G show some ways neurons can take the wrong path and/or find the wrong target.

the same few mutants,...there are now so many fascinating ones."

Among the most exciting mutants to emerge from the screen are those that address one of neurobiology's great mysteries: how embryonic nerve fibers find the correct route to their targets in the brain. These mutants have been identified by a team led by developmental neurobiologist Friedrich Bonhoeffer, a colleague of Nüsslein-Volhard's at Tübingen. For over a decade, Bonhoeffer has used isolated pieces of rat and chick embryonic tissues to study how axons originating in nerve cells in the retina find their way to the brain's visual center, where they touch down and inervate a structure called the tectum. This "termination" is very precise: Axons from the lower part of the right retina attach to the upper part of the left tectum; axons originating in the top of the left retina find their way to the bottom of the right tectum. The axons continuously "read" their position along the tectal surface, probably by detecting concentration gradients of specific "guidance molecules." But no one has been able to identify the molecules involved, says Bonhoeffer, "because of the crude, impure experimental systems we work in."

Realizing that zebrafish might offer a better alternative, Bonhoeffer jumped at the chance to piggyback onto Nüsslein-Volhard's genetic screen and search for zebrafish mutants with abnormal retino-tectal connections. Together with Herwig Baier, Stefan Klostermann, and Torsten Trowe, Bonhoeffer developed an ingenious mass-screening technique. First, fixed embryos are embedded in agarose and then loaded onto a microinjection device that deposits different fluorescent dyes in each of two specific regions of the eye, staining nearby nerve fibers. Under the microscope, the researchers can then see whether the stained axons have taken the correct route and reached the right target.



SOURCE: TORSTEN TROWE AND FRIEDRICH BONHOEFFER

Of 160 mutants isolated by Bonhoeffer's team, the most diverse group is the so-called pathfinding mutants, where the retinal axons take the wrong route from eye to tectum. Depending on the mutant, some axons get hopelessly lost, while others still find the right path to the right target. In another group, the termination mutants, the nerve fibers reach the tectum but inervate the wrong site; some, for example, fail to distinguish front from rear, others top from bottom. One of the termination mutants has a striking phenotype: The fish swim upside down (although this remarkable aberration could be due to a second mutation).

After Trowe described the range of mutant phenotypes at a recent symposium at the Cold Spring Harbor Laboratory in New York entitled "Zebrafish Development and Genetics," the audience broke into spontaneous cheers, says Sargent of NICHHD, who calls Trowe's presentation "one of the most exciting[,]...scientifically uplifting talks I ever heard." Adds Caltech's Fraser: "For every milestone, every checkpoint in the development of the [retinotectal] system, [they] have mutants affecting it."

The Tübingen mutants aren't the only ones wowing the field. Driever's group at the Massachusetts General Hospital (MGH) is conducting a separate, somewhat smaller, saturation mutagenesis screen. A former student of Nüsslein-Volhard, he uses similar husbandry techniques and so far has screened 800,000 embryos and found 600 mutants. And as befits his hospital-based lab, he's spreading the word to another group of potential zebrafish converts: medical researchers. Together with cardiologist Mark Fishman, also of MGH, Driever has collected more than 100 mutants with heart defects that may bring insights into human heart function. Some defects are structural (such as a missing atrium or improperly formed valves); other mutants have apparently normal heart structure but show functional defects (such as irregular or abnormally slow heartbeats).

Despite the rave reviews with which the scientific community is greeting the zebrafish mutant screens, Nüsslein-Volhard and Driever stress that the difficult work of identifying and cloning the genes that cause the mutant phenotypes and analyzing their normal function is only now beginning. Only a year ago the cloning step in particular looked like a huge problem, because, as a new model system, zebrafish lacked genome maps and the other tools that make it possible to clone new genes. But that's changing, thanks largely to researchers who are making genetic linkage and physical maps of the zebrafish genome.

Perfecting the technique for raising zebrafish was "absolutely crucial."
—Christiane Nüsslein-Volhard

Last month, John Postlethwait and his colleagues at the University of Oregon published a first genetic map of the zebrafish (*Science*, 29 April, p. 699). Howard Jacob of MGH is taking a slightly different approach, which will refine the map over the next year. Meanwhile, Postlethwait's team is developing high-resolution techniques for mapping zebrafish genes to within 100,000 base pairs. From there, researchers should be able to isolate individual genes by searching through the corresponding DNA fragments from zebrafish DNA libraries that are being assembled into a physical map in Hans Lehrach's lab at the Imperial Cancer Research Fund in London. As a result of these rapid advances, the "consensus seems to be that the cloning won't be nearly as big a problem as people thought it would," says Jim Smith, a *Xenopus* developmental biologist at the National Institute for Medical Research in London, who recently started working on zebrafish.

And when they have cloned genes in hand, vertebrate developmental biologists will at last be in a position to emulate their colleagues working on *Drosophila* by assembling a molecular picture of early development in a vertebrate. Driever predicts that putting together such an elaborate jigsaw puzzle will require a decade or more of solid work. But after years of frustration tinged with envy, researchers in the field are delighted that the new mutants and fast-developing genome maps should put them in a position to begin assembling that picture. Says Caltech's Fraser: "This opens a new era."

–Patricia Kahn