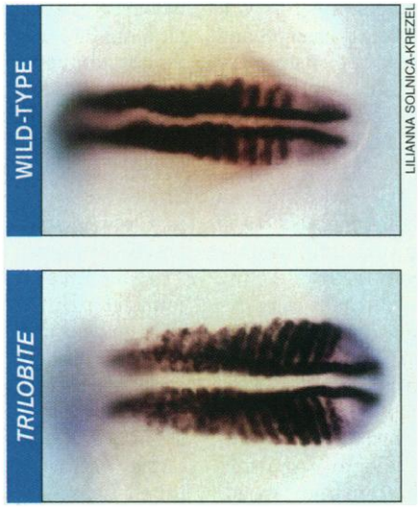
A New Embryo Zoo

A host of new mutations that disrupt development in the zebrafish will help identify the genes that guide vertebrate embryos as they unfold from featureless cells into specialized organs and tissues

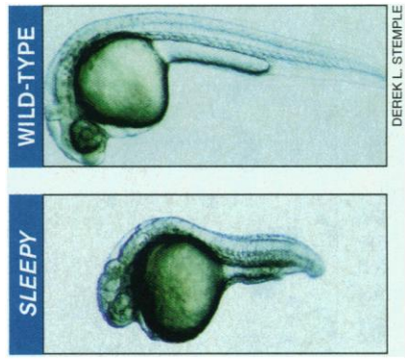
One of the most compelling experiments in developmental biology began in the late 1970s, when developmental geneticists Christiane Nüsslein-Volhard and Eric Wieschaus randomly mutated the DNA of thousands of fruit flies and then screened their offspring for embryonic defects. They opened a door on the genetic cascades behind an array of once-mysterious developmental processes and won a Nobel Prize for their efforts. Now, Nüsslein-Volhard and colleagues have done it again, this time on the vertebrate side of the animal kingdom. By applying their technique to the zebrafish, they have generated a bizarre bestiary of new vertebrate mutants and set the stage for progress in developmental biology for years to come.

After screening more than a million zebrafish embryos for developmental defects, two teams of geneticists, led by Nüsslein-Volhard at the Max Planck Institute for Developmental Biology in Tübingen, Germany, and Wolfgang Driever at Boston's Massachusetts General Hospital (MGH), have just published their first major findings, filling the entire December issue of the journal *Development*. They found congenital flaws in every stage of development, ranging from brainlessness to taillessness, from bigheartedness to two-heartedness, and from crazed swimming to immotility. To describe the roughly 2000 new mutants, they have raided the pages of fairy tales, cookbooks, and science fiction, choosing such namesakes as *spock* and *space cadet*, *bouillabaisse* and *chardonnay*, and all seven dwarves, from *sleepy* and *bashful* to *dopey* and *doc*. But despite the whimsical names, the science is profound: These faulty fish are expected to yield fundamental insights into vertebrate embryogenesis, the process by which a condensed genetic recipe unfolds into a body with fully specialized organs and tissues. "To depict the embryo in terms of networks of gene expression is a very ambitious goal," says Hazel Sive, a molecular embryologist at the Whitehead Institute for Biomedical Research in Cambridge, Massachusetts. "But this is a very exciting set of mutants."



An early flaw. In *trilobite*, a gastrulation-stage mutant, cell movement is curtailed.

By isolating the disrupted genes themselves, the researchers hope eventually to reconstruct the web of gene transcription and protein action that guides embryogenesis. "Our mutations affect some of the very earliest processes in development," says Nüsslein-Volhard. "We hope this will open up new areas of research." And because throughout evolutionary history organisms have tended to modify rather than replace successful genetic mechanisms, the new insights in zebrafish are bound to yield clues to embryonic development in humans.



Support staff. *Sleepy* has a malformation of the normally sturdy notochord.

The zebrafish screen closely mimics the "saturation screen" of fruit fly (*Drosophila melanogaster*) embryos that Nüsslein-Volhard and Wieschaus did years ago. They used a chemical mutagen to disable as many of adult flies' key developmental genes as possible, then sifted through thousands of descendant embryos to pick out the mutants. This work uncovered many of the molecular events behind fly development and won the pair a trip to Stockholm in 1995 (*Science*, 20 October 1995, p. 380).

But while many of the fly's genes turned out to be generic—used across the animal kingdom—these studies yielded limited information about the development of structures unique to vertebrates. To learn about the development of a complex nervous system or internal organs such as kidneys, for example, researchers needed a comparable set of vertebrate mutants. The screening method, however, requires fast-breeding subjects that produce readily observable embryos. No vertebrate lab animal seemed to fit the bill—until Nüsslein-Volhard turned to the striped, inch-long zebrafish *Danio rerio*.

In 1993, after 5 years spent fine-tuning aquarium accommodations for some 350,000 of the freshwater fish, her group began exposing hundreds of males to a chemical that can disable genes in the fish's sperm. They then examined the embryos produced by the descendants of the exposed fish. At the same time, Driever, a former student of Nüsslein-Volhard, started a parallel, slightly smaller screen in Boston.

Because researchers at the University of Oregon in Eugene, including Chuck Kimmel and the late George Streisinger, had already documented many details of normal zebrafish development, the two groups could easily pick out the mutant embryos. Even so, the screen was a colossal task. "We had 10 or 12 people working 60 to 70 hours a week for 9 months," recalls Mary Mullins, who moved from Nüsslein-Volhard's lab to the University of Pennsylvania.

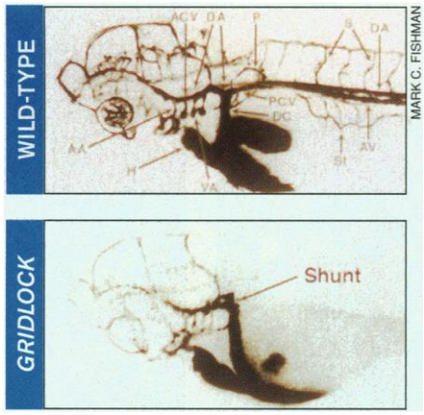
Now that the researchers have built a bank of these mutant fish stocks—complete with records of the mutants' pedigrees and the frozen sperm of their carrier fathers as backup—the real genetic analysis can begin. A clear understanding of the molecular mechanisms disrupted by the mutations is a long way off, because simply cloning the affected genes could take years. But if the *Drosophila* screen is any guide, many of these genes will bring to light previously unimagined developmental pathways, says Chris Wylie, a developmental researcher at the University of Minnesota and editor in chief of *Development*.

Below, we offer a brief sampling of these freaky fish and the developmental tales they may have to tell:

Gastrulation gone astray

In its earliest hours, the zebrafish embryo is a featureless bowl of cells cupping a large central yolk cell. Between 6 and 24 hours after fertili-

zation, however, cells from two sides of the bowl converge on its equator, forming a tubelike body that divides into segments called "somites" and elongates in a C shape around the yolk. Perhaps the most crucial phase of embryogenesis, this process of gastrulation sets up the embryo's multilayered body plan and helps it tell head from tail (anterior from posterior) and stomach from back (dorsal from ventral).



Jammed up. *Gridlock* impairs the embryo's blood circulation at the point labeled "shunt."

Biologists believe that molecular messages between cells orchestrate this complex series of events, but so far they have detected only a few such signals. The zebrafish screen has opened a window on this early developmental stage by revealing many gastrulation-impaired mutants, such as half *baked* and *bozozok* (Japanese for "arrogant youth on motorcycles"). "The mutants can uncouple certain events and processes, with each one giving us a different molecular entry point into the cellular mechanics involved," explains geneticist Lilianna Solnica-Krezel of Vanderbilt University in Nashville, Tennessee, a former member of Driever's lab.

For example, in two mutant strains discovered by the Driever lab—one called *trilobite* and another called *knypek*, Polish for "short"—cells don't converge fully toward the notochord, a rodlike support structure that defines the anterior-posterior axis during gastrulation and is later superseded by the backbone. Cells also fail to extend fully around the yolk. The resulting embryos have a flattened appearance that indeed resembles a trilobite. Solnica-Krezel speculates that the mutations affect either the signals that help cells navigate or part of the cytoskeletal machinery used by cells to change shape; she's working on cloning the genes and tracking abnormal cell movements in mutant embryos in order to test these ideas.

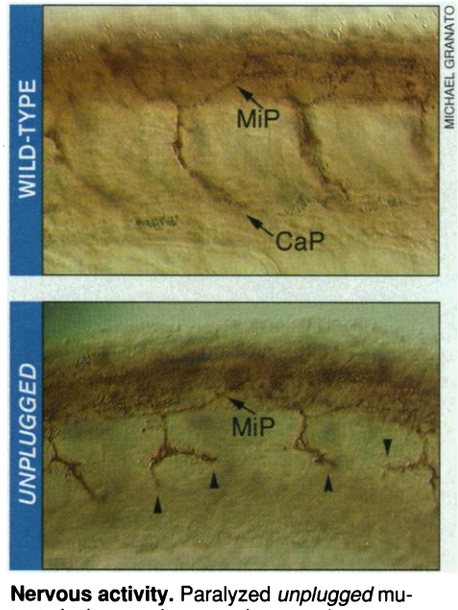
A flat in the notochord

Other mutations affect the notochord itself or disrupt one of its crucial tasks: directing the differentiation of nearby cells. For example, the notochord is stiffened by large bubbles or "vacuoles" in each of its cells, much as air stiffens a balloon. This allows the notochord to brace the embryo as its posterior half lengthens and pulls free of the yolk cell several hours after gastrulation. But in one group of mutants, including *sleepy*, *grumpy*, and *bashful*, these vacuolated cells never appear, and the embryo ends up with a wiggly, shortened tail.

Derek Stemple, a postdoc in Driever's lab, believes that the mutations might be disrupting crucial steps in the program that causes notochord cells to differentiate. These mutations may also help reveal how the notochord instructs neighboring tissues to differentiate. For example, in *sleepy*, *grumpy*, and *bashful*, the somites that normally form a chevron pattern on either side of the notochord are malformed. By using antibody staining, Stemple found that the cells in these chevrons haven't developed into muscle precursor cells, which normally build the somites. That suggests that the notochord is needed to send a signal to nearby cells directing them to become muscle precursors. "There are a host of genes" expressed in the notochord region, Stemple says, and the mutants will help researchers determine how and in what sequence each gene contributes to the formation of the notochord and somites.

Out of circulation

The notochord is only a temporary scaffold for the zebrafish embryo, providing structural support while the skeleton and other permanent structures, such as the heart, liver, kidney, and circulatory and digestive systems, are assembled. The Boston and Tübingen groups' genetic blitzkrieg created a welter of mutations in these systems too.



Nervous activity. Paralyzed *unplugged* mutants lack normal neuronal connections.

Some of these later occurring mutants, which include *breakdance*, *casanova*, and *meltdown*, are already yielding clues to related mutations in human embryos.

One mutant identified by Driever's lab, for example, lacks blood flow in its tail. To investigate the defect, Driever, lab member Brant Weinstein, and MGH cardiovascular researcher Mark Fishman devised a "microangiography" method to track circulation through the tiny embryo's blood vessels. They discovered a blockage at a key intersection, where two major arteries normally merge into a single vessel, and they named the mutant *gridlock*.

That glitch caught Fishman's eye because it resembles coarctation of the aorta, a common and deadly birth defect in humans. In coarctation, a ledge of tissue obstructs blood flow in the descending aorta near the heart, a region that has its embryonic origins at precisely the point that *gridlock* jams circulation in zebrafish. "It may be that this is a gene that, when mutated, gives this exact defect" in circulatory-system patterning in both species, Fishman says.

Axons adrift

It was odd behavior, not fractured anatomy, that made other mutants stand out from the crowd. Some mutants, such as *techno trousers* and *backstroke*, twitched uncontrollably or swam in circles, while others could hardly move at all. Such erratic behavior may be linked to subtle defects in the nervous system, and these mutants may offer biologists a handle on vertebrate neuronal development, says Michael Granato, another former member of Nüsslein-Volhard's lab who is now at the University of Pennsylvania.

For example, one mutant with locomotion problems, *unplugged*, suffered virtual paralysis at 24 hours after fertilization. The mutant had no obvious muscle defects, so Granato suspected a wiring problem—perhaps an error in one of the nerve endings, or axons, that grow out from the spinal cord to innervate specific target muscles. When he stained *unplugged* embryos with an antibody that sticks to motor neurons, he saw that one axon extending into the ventral half of each somite formed aimless branches, as if it had lost its way. It's possible, Granato says, that the affected gene encodes a protein similar to several already known to be used for axonal guidance and target recognition in *Drosophila* (*Science*, 29 March, p. 1807).

Granato, like a host of other researchers, is already busy cloning and analyzing his favorite mutant genes. That task is likely to go on for years. But the magnitude of the job hasn't dimmed the wonder that vertebrate developmental biologists are feeling as they explore their new mutational zoo.

–Wade Roush