DEVELOPMENTAL BIOLOGY

Zebrafish Embryology Builds Better Model Vertebrate

ILLUSTRATION: K. SUTLIFF SOURCE: H. SIVE

The trouble with studies of vertebrate development, molecular embryologist Hazel Sive says, lies in the leading animal model: the fromoshken. And she holds one up—a fanciful drawing that's part frog, part mouse, part zebrafish, and part chicken. "There's no model vertebrate where one can study every aspect of development," Sive explains. Frog and chicken embryos are great for grafts and other manipulations to reveal when and where tissues such as nerves and skin first arise, but researchers must turn to the many mutations of mice or zebrafish to learn how genes affect development. Yet in those animals, scientists haven't been able to isolate embryonic tissues with which to study development. So, says Sive, "we're putting together little bits and pieces from different organisms into this vertebrate—this fromoshken—that is neither one thing nor another." And researchers fear that piecemeal ideas about development may be just as fanciful as the organism.

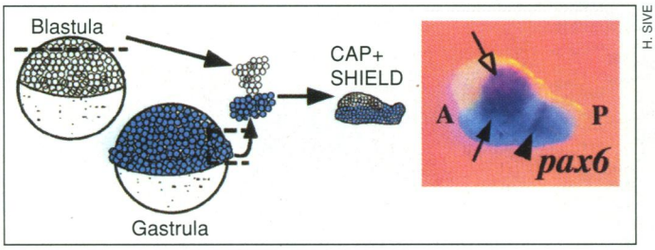
Outdated "fromoshken." Frog-mouse-fish-chicken combo.

The fanciful, however, is now giving way to the fish. Sive and her colleagues at the Whitehead Institute for Biomedical Research in Cambridge, Massachusetts, report in the June issue of *Development* that they've brought experimental embryology home to the master of mutations, the zebrafish. They've perfected a method for whittling tiny clusters of cells, or "explants," from early zebrafish embryos and growing them in vitro. And the payoff—actually two payoffs—is very big.

First, the technique has provided much-needed reassurance that the zebrafish, a highly touted model for vertebrate development that would lose value if it followed some odd developmental course, is a good model after all. Sive's team has watched two explants from normal fish exchange signals that trigger the formation, or "induction," of neural precursor cells in tissue from the embryo's future brain—just as cells in frogs and other vertebrates do. "Sive is providing the data we need to link findings in amphibians, zebrafish, chickens, and mice," says Scott Fraser, a developmental neurobiologist at the California Institute of Technology.

Second, the new zebrafish embryology should enable scientists to capitalize on the growing library of zebrafish genetic mutations and learn with unprecedented precision how they disrupt the formation of developing tissues. Says developmental biologist John Postlethwait of the University of Oregon: "This marriage of approaches is going to be incredibly powerful."

Zebrafish have been elusive embryologically because researchers have lacked a way to probe interactions between tissues under controlled conditions, away from the confusing clamor of biochemical signals that courses through the intact embryo. Groups of cells from the early embryo, or blastula, quickly fall apart and die when removed, because they are cut off from the embryo's central yolk cell, which provides both food and footing. The problem was especially vexing for Sive and study co-authors Charles Sagerström and Yevgenya Grinblat, who wanted to home in on the earliest signals that divide the embryo's developing central nervous system into anterior (head) and posterior (tail) portions.



H. SIVE

Telling head from tail. Zebrafish embryo "explants" show that *pax6* (a marker of future brain tissue) is activated in one tissue type by another.

From earlier work on frog embryos in Sive's lab and others, the group suspected that this subdivision was based on positional cues delivered by cells from an area known as the "embryonic shield," a thickened region on one side of a later embryonic stage, the gastrula. Shield cells come in contact with many other cells in the embryo. And shield explants—which Sive's group found were large enough to survive on their own in vitro—developed their own anterior-posterior pattern as they aged: They grew a posterior knob, for instance, and expressed the gene *pax6*, a marker of future forebrain and hindbrain tissue, only in one stripe at the anterior end of the explant and in another stripe midway toward that knob.

Sive and co-workers wanted to see if the zebrafish shield provides a template for anterior-posterior differences elsewhere in the embryo. But to do so they needed a clean slate: an explant from another area that could also survive in vitro and whose cells, as far as the researchers could tell, had not yet learned head from tail. They chose the animal cap, a cluster of about 50 cells at one pole of the blastula. After nearly a year of experimentation, the researchers discovered a way to keep animal-cap explants alive for the duration of their experiments. A group of 10 caps assembled into a 500-cell blob, they found, could grow its own epithelial coating, which helps hold the cells together and provides a sac to hold in secreted growth factors and extracellular nutrients. (Similar methods will enable the researchers to culture explants from many other parts of the embryo, Sive hopes.)

With their slate ready, the group then applied the shield template. The scientists attached animal-cap conglomerates to the shield explants and watched what happened. After 9 hours, the researchers saw, the same gene markers present in the anterior half of each shield explant, including *pax6*, also appeared in the adjacent region of the animal caps. Posterior markers in the shields, however, never turned on in the animal caps. "What that suggested to us is that we were getting induction, but it was only anterior induction," says Sive. Additional signals may be needed in order to activate expression toward the tail, she speculates.

While incomplete, the picture of multistep cell fate determination the Whitehead study has produced is already detailed enough to show that "inductions in zebrafish look very similar to those in other, higher vertebrates," including frogs and mice, says Wolfgang Driever, a developmental biologist at Massachusetts General Hospital. And that's welcome news for those involved in the 7-year-old effort by several labs to generate mutant strains with interesting developmental defects (*Science*, 13 May 1994, p. 904). Says Postlethwait: "It is certainly comforting to find that out."

Now scientists hope to learn exactly how the mutant zebrafish genes they have cataloged exert their effects. In one mutant zebrafish strain that lacks a brain, for example, explant experiments may help determine whether the defect arises because the signals that trigger brain tissue formation are missing, or because the cells that normally give rise to brain tissue simply can't respond. The explant technique, Sive says, thus promises to bring embryological techniques together with modern developmental genetics "in a way we've never been able to do before in a single vertebrate." It also promises to put the fromoshken out to pasture, pond, and chicken coop.

–Wade Roush