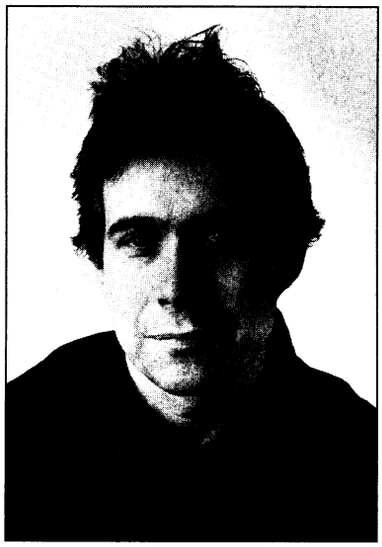
including muscles, bones, blood.

The process whereby a mass of undifferentiated cells is set onto a path that leads to a few types of specialized tissues is known as induction, and it takes place as a kind of chain reaction. For example, mesoderm forms a structure called the notochord, which induces nearby ectodermal cells to give rise to a structure called the neural tube. The neural tube goes on to develop into the brain and spinal cord, as well as inducing other ectodermal cells to elaborate the lens of the eye. The first link in this chain is the event that leads tissues in the developing blastula



U.K. Medical Research Council

Signal processor. *Has Jim Smith found a key chemical signal that induces embryonic tissues to differentiate?*

to become mesoderm, ectoderm, and endoderm—and how that happens has long been a subject of intense inquiry.

In the 1920s, two German embryologists, Hans Spemann and Hilde Mangold performed a classic experiment aimed at finding the first link in the chain. They transplanted a small group of cells from a specific region of one blastula to another that was just undergoing gastrulation. The result was a complete secondary embryo centered on the transplant site. This region, which Spemann called the primary organizer (and which modern biologists call Spemann's organizer) is the only group of cells capable of initiating a complete embryo, and developmental biologists want very much to know what the signal is that Spemann's organizer is sending out. Again, molecular biology is beginning to come up with some answers.

Jim Smith, a molecular embryologist at the Medical Research Council's National Institute of Medical Research in north London, thinks the first signal is probably a substance called activin, which is a peptide growth factor. Smith initially isolated activin from culture medium in which *Xenopus* cells were growing. He then showed that it could induce cells from the animal pole (which would otherwise become ectoderm) to become mesoderm.

That was a good start in uncovering the signal from Spemann's organizer. But, Smith reasoned, if activin is indeed that signal, it should be able not just to create mesoderm, but also to mimic some more subtle actions of the organizer, which has a complex effect on tissues around it. The cells closest to the organizer become endoderm; those slightly farther away become mesoderm; those farthest away become ectoderm. Along with most embryologists, Spemann assumed this was the result of a concentration gradient of the form-giving signal. So Smith put cells from the animal pole into culture with different concentrations of activin.

The results were remarkable—although not completely clear-cut. At low concentrations, activin induced epidermis, an ectodermally derived tissue. A concentration 50% higher triggered muscle and notochord. Another increase turned the cells into mesoderm capable of changing ectoderm into neural tube cells. These results suggest there are two sharp thresholds of activin activity, and that the molecule is capable of inducing both ectoderm and mesoderm in different concentrations.

Activin thus seems to be able to mimic some key aspects of the activity of Spemann's organizer. But Smith has a ways to go before he can say he's identified the long-sought chemical messenger. For one thing, intermediate concentrations of activin yield a combination of muscle and notochord. That is problematic because notochord is the most dorsal of mesodermal tissues; muscle is more ventral. Hence there cannot be a simple correspondence between activin concentration and dorso-ventral position in the embryo—so perhaps more than one signal is involved.

A somewhat knottier problem is that the developing embryo seems to contain no activin—at least not until the blastula stage, by which time the signal from the primary inducer has already been received and acted on. In his talk, however, Smith hinted that he has found traces of activin in the earlier embryo. But he refused to show slides with his data, remarking that he might be wrong and "if I show slides, you'll remember it."

But whether or not Smith can find conclusive traces of activin in the early embryo, it seems clear that a second great revolution in embryology is well under way. After the descriptive progress of the last century, researchers are now beginning to be able to decipher the movements of molecules that guide the tissues as they move and flow to form a complete organism.

■ JEREMY CHERFAS

Zebrafish:

Eugene, Oregon—IS A TINY, ZEBRA-striped fish from the Ganges River going to be the answer to a developmental biologist's prayers? It just may be, if the lofty expectations of a growing band of researchers pan out. They believe it may eventually prove to be as good a system for studying vertebrate development as the fruit fly ***Drosophila*** and the roundworm ***Caenorhabditis elegans*** have been for invertebrates.

What ***Drosophila*** and ***C. elegans*** have going for them is a powerful combination of embryology and genetics. Researchers can not only see the developing embryos of these animals, but they can also induce mutations in the embryos and observe how those mutations perturb normal development. As a result, invertebrate developmental biology has made great strides in the last decade.

But there hasn't, until now, been such a system for vertebrates, which represent the next great challenge for developmental biologists. Mice have long been popular for genetic study, but their embryos are hidden in the uterus. The African clawed frog ***Xenopus laevis*** has been a good subject for embryology (see story on p. 33), but frogs are slow to breed and therefore not good subjects for genetics.

But the zebrafish—a 2-inch freshwater fish popular in home aquariums—may just be the creature developmental biologists have been seeking. What it provides is "the possibility of combining an excellent embryology with a fairly good genetics," says ***Drosophila*** developmental biologist Jose Campos-Ortega, who has begun studying zebrafish in his lab at the University of Cologne, West Germany.

The interest of people like Campos-Ortega is new and marks a turning point for zebrafish research, which until recently was confined almost exclusively to the University of Oregon, where the late geneticist George Streisinger got the system started 20 years ago. Now zebrafish research is on the verge of taking off: a dozen or more laboratories—including some international powerhouses—are jumping on the bandwagon. That new interest was reflected in a workshop held last month (appropriately enough in Eugene), where members of the budding zebrafish community discussed what is needed to get their model up to speed. The consensus at the conference: Hard work lies ahead—particularly in developing the specific genetic tools that will make the zebrafish truly productive—but the payoff will be worth it.

Streisinger, a phage geneticist, could not

Swimming into the Development Mainstream

have fathomed all the zebrafish's attributes when he picked it. All he wanted was a good vertebrate subject for genetic research. He was attracted by the fish's brief (3-month) generation time and prolific egg production. Over the next decade, Streisinger patiently devised techniques to raise and breed zebrafish in the lab, collect its eggs and sperm, and mutagenize its DNA. Meanwhile, Streisinger's colleague Charles Kimmel discovered the fish's other great advantage—transparent, rapidly developing embryos that are ideal for developmental study.

Although the use of zebrafish as a developmental model is only now picking up steam, Kimmel and Judith Eisen, also of Eugene, have already provided some tantalizing evidence of how zebrafish embryology and genetics can be combined to address developmental questions. One example comes from a mutant called spadetail, in which cells that normally form segmented muscle tissue bunch up in the embryonic tail instead. Eisen showed that some nerve cells in spadetail mutants also grow abnormally, suggesting that the gene mutated in spadetail is necessary for normal development of both nerves and muscles.

But which cells express the gene, and which cells respond to it? In a step toward answering these questions, Eisen and Robert Ho, a postdoc with Kimmel, have transplanted cells between embryos. Ho showed that muscle precursor cells from the spadetail mutant still head for the tail when they're put into a normal embryo; the normal environment can't correct for the cells' lack of an intact spadetail gene. But when Eisen moved developing nerve cells from spadetail mutants to normal embryos, the nerve cells grew correctly. They seem to respond to the product of the spadetail gene, but don't need to make it themselves.

"That's what zebrafish is all about," says workshop participant William Harris, who studies *Xenopus* at the University of California, San Diego. "[These] combinations of techniques allow you to do a biological experiment you can't possibly do in any other [vertebrate] organism."

But one or two interesting mutants aren't enough for a productive model. To understand the cascade of signals involved in any developmental process requires technical tools: the ability to make mutants in every step of the process, a genetic linkage map of the fish's 25 chromosomes, and efficient methods for cloning interesting genes, such as the ability to tag them with foreign DNA.

Zebrafish researchers don't yet have any of these things. "As a young person looking at it now, I can say there are so many tools that we need [and] don't have that it's daunting," says Carl Fulwiler, a postdoc who is setting up zebrafish studies with molecular biologist Walter Gilbert at Harvard.

This year's model. *The zebrafish is an intriguing new model system for embryonic development.*

While the tools won't come overnight, the zebrafish is now attracting researchers who have the expertise needed to develop them. Christiane Nusslein-Volhard, a *Drosophila* geneticist at the Max Planck Institute in Tubingen, West Germany, is well known for having found mutations in nearly all the genes responsible for early pattern formation in *Drosophila* embryos and using those mutants to work out the genetic logic governing the process. Drawn to zebrafish by their "truly wonderful" embryos, she plans a similar genetic survey in the fish.

That survey won't be a simple matter: it will mean treating large numbers of zebrafish with mutagens and then looking at tens—possibly hundreds—of thousands of embryos to find the ones with mutations that throw early development off the track. Such a mutant screen can't be done now since existing facilities can't hold enough fish. But Nusslein-Volhard is experimenting with ways to grow the fish more compactly and streamline their handling.

The second desideratum—a genetic map—may soon be in the works as well. Geoffrey Duyk, a human geneticist at UC San Francisco, is experimenting with the possibility of using DNA polymorphisms, stretches of DNA that differ among individuals, to construct such a map. A nifty trick developed by Streisinger for making fish with two identical sets of chromosomes will simplify the mapping, says Duyk.

But cloning genes will be tough even with a good map, and some workshop participants argued that zebrafish researchers need a means of using foreign DNA to interrupt and thus mutate genes. The inserted DNA can later serve as a marker for cloning the gene. Monte Westerfield, the third member of the Oregon zebrafish triumverate, has succeeded in introducing foreign DNA into the zebrafish genome. But the efficiency of DNA insertion must be increased if DNA is to be used as a mutagen. In *Drosophila* that efficiency boost was provided by transposons—or jumping genes—found in a wild fly strain. Whether a useful transposon exists in zebrafish is not known, but several years ago the Eugene group noticed a high natural mutation rate in a zebrafish strain from Singapore, a sign that the strain may harbor a transposon.

To build a genetic system from scratch is no mean feat, and it is too early to say how well the newly enlarged zebrafish community will rise to these challenges. Workshop participants didn't agree on many of the details of how to proceed, such as which mutation schemes are best, or to improve DNA insertion methods. But differences of opinion may be the best thing for the field at this stage, says David Grunwald, who is beginning to search for developmental mutants in his lab at the University of Utah. "I would argue for a diversity of approaches," says Grunwald. And with the growing interest in zebrafish, that is just what the field is likely to get.

■ **MARCIA BARINAGA**