Embryology Gets Down—to the Molecular Level

The chemical messengers underlying the complex morphogenetic movements of the embryo are beginning to come to light

Heidelberg, West Germany—IN ONE SENSE embryology hasn't advanced much in the last 100 years. By the middle of the 19th century much of the choreography in the fertilized egg—the movements of cells and tissues, the complex foldings and twistings that lead to a differentiated organism—had been precisely described in many species. Yet no one knew what chemical signals passed between cells and tissues to choreograph them so neatly.

In the past few years that has begun to change. With the tools of molecular genetics in hand, developmental biologists have finally begun to tease out the molecules that carry signals to the dancing tissues of the embryo. Among the fruits of that work were a couple of intriguing papers presented at the recent annual symposium of the European Molecular Biology Organization here. The symposium, devoted to vertebrate development, made clear that in two key areas—information encoded in the cytoplasm of the egg and the induction of differentiated tissues—some molecular mechanisms and messengers are indeed coming to light.

The information encoded in the egg's cytoplasm is crucial, because although geneticists are fond of saying that the genome contains the entire blueprint for an individual, the embryologist knows that's not true. The genome of the fertilized egg doesn't go to work until the egg has already taken several fateful steps down the road to a new individual; clearly the genome can't direct those prior steps. Where does the genetic information for the first steps come from? The general answer, which has long been known, is that it comes from the mother, in the form of molecules stored in the egg's cytoplasm. But which molecules? And how do they work?

Developmental Biology

The fast-moving world of developmental biology has been even busier than usual in the last few weeks. In Heidelberg, the European Molecular Biology Laboratory's annual meeting was devoted to vertebrate development, as Jeremy Cherfas reports. A few days earlier and halfway around the world—in Eugene, Oregon—some of the same scientists had met to consider the zebrafish, an organism that may one day be the vertebrate *Drosophila*, as Marcia Barinaga reports on page 34.

Chris Wylie, an embryologist at Cambridge University's Department of Zoology, has been getting some preliminary answers to those questions in work with a favorite pet of developmental biologists: the African clawed frog, *Xenopus laevis*. The large *Xenopus* egg is divided into two parts: a dark animal pole and the lighter, larger, vegetal pole. The animal pole eventually forms the tadpole, while the vegetal pole supplies the yolk that nourishes it. Development begins with the first cleavage of the fertilized egg in two, which divides the poles and confirms the axis of the embryo's future growth. These are among the changes that take place before the genome is turned on, and Wylie is interested in what controls them—particularly the location of that first cleavage.

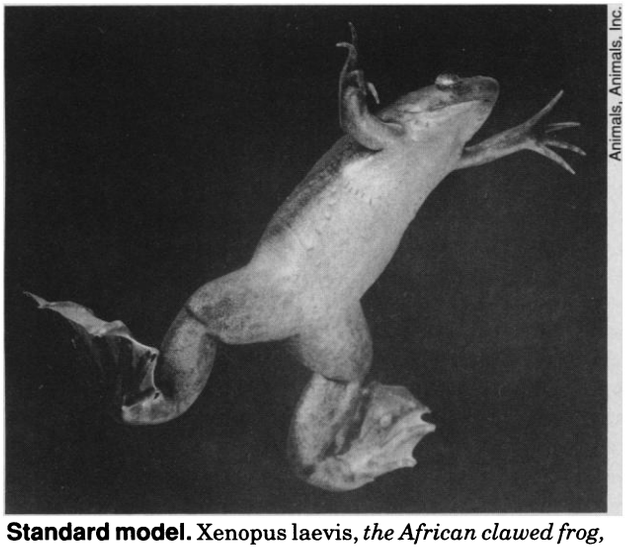
His suspicion is that the cytoskeleton—the framework of large molecules that give the egg (like other cells) its form—plays a large role. In particular, he thinks one of the chief cytoskeletal proteins, vimentin, does much of the work. Wylie's experimental strategy, as he explained at the EMBO meeting, is to knock out vimentin and see what effect that has on the early cleavage pattern in the fertilized egg. Knocking out vimentin is done by blocking the messenger RNA that carries the code for the protein; the blockade is achieved with short strands of DNA complementary to vimentin messenger RNA, which, when injected into the egg, create partially double-stranded messenger molecules.

The double-stranded stretches attract the attention of the enzyme ribonuclease H, which begins to separate the strands. Other enzymes then come along and nibble at them. The result, says Wylie, "is a very clean experiment. All you have left is a jumbled mass of nucleotides": in short, an egg deprived of vimentin messenger RNA—and of vimentin.

What effect does this blockade have on the egg? High doses of the anti-vimentin DNA completely blocked the first cell division. Intermediate doses resulted in abnormal cleavage patterns, particularly in the vegetal pole, but low doses seemed to have no effect: the tadpoles that developed were apparently normal. Internal examination, however, revealed that there were very few germ cells in these tadpoles. This result is not unreasonable, since antibodies to vimentin (and hence presumably vimentin itself) are concentrated in the region of the egg that ultimately forms the eggs or sperm in the adult.

These results, which drew much attention from the 300 scientists in the audience here, suggest that vimentin does have a key role in events before the genome of the new creature begins operation. Yet the mechanism by which vimentin has these effects is far from clear. The protein is definitely not a mechano-enzyme, that is, one responsible for moving things around in the cell. But vimentin could have a role in positioning components within the egg. "It's most likely scaffolding that holds things in the right place," Wylie says. Vimentin could also have a function in binding some as yet unknown molecule. It will take considerably more work, however, to find out whether vimentin is indeed a binding protein and, if so, what its binding target is.

After the early changes that Wylie's work concentrates on, the really elaborate choreography of the *Xenopus* embryo begins. Early cleavages create a solid ball (the morula), then a hollow ball (the blastula). In the next stage—called gastrulation—the hollow ball folds in on itself, in the process creating three layers of cells: ectoderm, mesoderm, and endoderm. Ectoderm goes on to form the skin and the nervous system; endoderm forms the inner lining of the gut and lungs; mesoderm forms everything else,

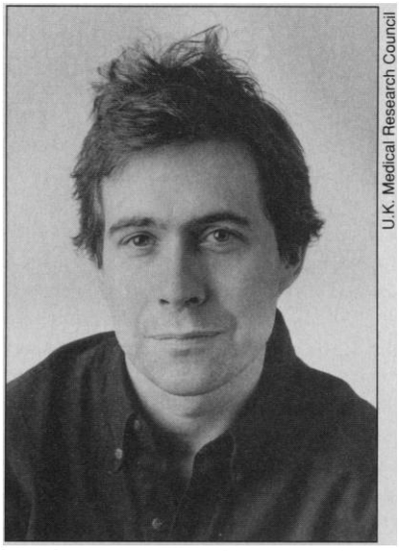


Animals, Animals, Inc.

Standard model. Xenopus laevis, *the African clawed frog, has long been used to study vertebrate embryology.*

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



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