How Embryos Tell Heads From Tails

Developmental biologists are beginning to work out the molecular and cellular details of how the vertebrate body plan is established

MORE THAN 70 YEARS AGO, GERMAN EMbryologist Hans Spemann embarked on a series of experiments that continues to intrigue his counterparts today, decades after his death. Spemann found that a specific small piece of tissue from the salamander embryo has remarkable generative properties. Transplanted to a second embryo, this piece, now known as the "Spemann organizer," causes the recipient embryo to develop a second head-to-tail axis. In extreme cases, the embryos have two heads.

Developmental biologists have spent almost the entire 20th century trying to figure out how the organizer brings about axis formation. Although much of the work has been done with amphibian embryos, axis formation is a key event in the development of all vertebrates, and the researchers hope that what they learn from amphibians will provide insight into what goes on in the higher vertebrates as well. Says one prominent student of the Spemann organizer, Randall Moon of the University of Washington School of Medicine in Seattle: "To some degree, it's been the Holy Grail of embryology for most of the century." But now the Grail may be in sight.

New experiments have begun to zero in on the genes that may underlie the organizer's activities. More than that, recent work in several labs—moving at a pace so rapid that models that seemed firm only a few months ago have already been left behind—has also begun to identify the chemical signals that lead to the very creation of the organizer. As a result, today's developmental biologists are at last beginning to understand the molecular and cellular basis of embryonic development as described by Spemann and other classical embryologists many decades ago. Says Moon: "It's turning into real cell biology."

To understand how the Spemann organizer forms, it's necessary to untangle the mysteries of how the mesoderm—the embryonic tissue where the organizer is located—forms. The mesoderm, which gives rise to muscle and blood, is the third of the embryo's three tissues to develop. The first two are the ectoderm, which gives rise to skin and nerves, and the endoderm, which produces the lining of the digestive system. The cells of the endoderm and ectoderm then interact to induce the formation of mesoderm.

As recently as the beginning of this year, developmental biologists thought they had a pretty good idea of how that happened. But the neat model they were developing has been upset by more recent results.

That model was suggested about 10 years ago by Jonathan Slack, Jim Smith, then a postdoc with Slack, and their colleagues at the Imperial Cancer Research Fund's lab in Oxford, England, based on studies of development in the frog *Xenopus laevis*. This is a convenient organism for studying development because the embryos are relatively large and sturdy enough to be manipulated in lab culture, and because they are color-coded. One half, the animal hemisphere, is dark colored; it is where the presumptive ectoderm develops. The other half, the vegetal hemisphere, is light colored; the presumptive endoderm develops there. Mesoderm induction occurs at the equator of the embryo with the Spemann organizer forming at the future dorsal side, where the animal's head and other anterior structures will eventually arise.

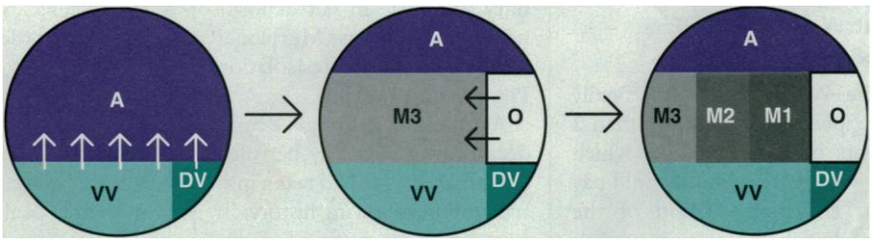
Embryologists have known for years that factors produced in the vegetal hemisphere induce mesoderm formation. What Slack and his colleagues proposed was that there are only two such factors, one for the dorsal mesoderm and the other to spark development of the ventral mesoderm, which gives rise to the embryo's posterior structures.

At the time, no one had any idea what the two factors might be. Then, a few years ago, evidence began to accumulate that they were peptide growth factors. Slack's group and also that of Marc Kirschner at the University of California, San Francisco, showed that basic fibroblast growth factor (bFGF), which has several activities including stimulating blood vessel formation, might also be the ventral-mesoderm inducing factor. And last year, several groups, including those of Smith, who by then was at the National Institute for Medical Research in London; Danny Huylebroeck of Innogenetics, S.A., in Ghent, Belgium; Makoto Asahima of Yokohama City University; and Douglas Melton of Harvard University, obtained evidence suggesting that the other factor—the one that induces the dorsal mesoderm and thus the Spemann organizer—might be one of the activins, which have been implicated in blood cell differentiation and are related to transforming growth factor beta.

The researchers showed, for example, that when animal hemispheres isolated from *Xenopus* embryos are incubated with activin, they form prominent head structures, as might be expected if activin contributes to Spemann organizer formation. By early this year, Melton says, "everyone believed that the signaling molecule [for inducing the dorsal mesoderm] was likely to be activin."

But even then there were some problems with the idea. For one thing, activin didn't seem to be made early enough in the embryo to initiate the formation of the dorsal mesoderm. And then Melton and Sergei Sokol, a postdoc in his lab, obtained results suggesting that the dorsal part of the *Xenopus* animal hemispheres was already committed to form head structures before they were exposed to activin. That result indicated that some other factor had already established the dorsal cells' fates, with activin affecting mesoderm development only later, if at all.

Then came a series of recent results that named a new candidate. In two papers in the



Early view of mesoderm induction. *At first just two factors from the* Xenopus *vegetal hemisphere (VV and DV) seemed to be involved, with the organizer (O) producing a third. But more recent work shows that it's not that simple...*

SOURCE: J.M.W. SLACK *ET AL., NATURE* 326, 197 (1989). ILLUSTRATION: D. DEFRANCESCO

15 November issue of *Cell*, one from Sokol and Melton, working in collaboration with Moon and his postdoc Jan Christian, and the other from William Smith and Richard Harland at the University of California, Berkeley, members of another gene family—called *Wnt*—were tapped as better at organizer induction than the activins.

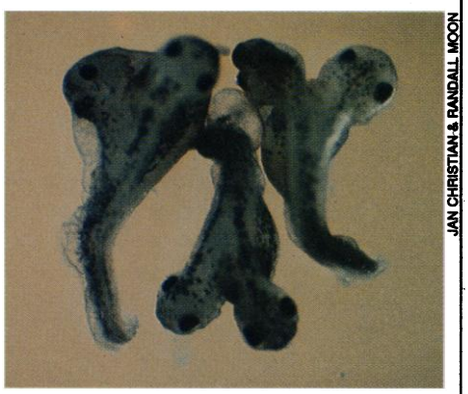
The two groups came to that conclusion by different routes. Moon was familiar with the *Wnt* genes as a result of previous work with Andy McMahon at the Roche Institute of Molecular Biology in Nutley, New Jersey. Work in several labs had implicated the *Wnt* genes in development. And about 3 years ago, Moon and McMahon found that if they injected *Xenopus* embryos with the messenger RNA that makes the Wnt-1 protein, the embryos developed double axes—just as Spemann's original embryos had. At the time, the researchers thought the Wnt protein merely caused the Spemann organizer to split, but the new work suggests that Wnts may have a much more fundamental role.

In their current experiments, Sokol, Christian, Moon, and Melton compared the effects of the messenger RNAs for Wnt-1 and Xwnt-8, and for activin, by injecting them individually into early *Xenopus* embryos. They found that when they injected Wnt-1 or Xwnt-8 RNA at the embryos' ventral side, away from the site where the Spemann organizer forms, the RNAs induced a complete second axis, producing two-headed embryos. "That's pretty neat," says Moon. "It tells us pretty certainly that a signaling pathway activated by Wnt is involved" in induction of the Spemann organizer. In contrast, activin RNA induces only a partial second axis, lacking such anterior features as eyes.

Smith and Harland, meanwhile, were going the gene cloning route. The development of the head-to-tail axis can be blocked in *Xenopus* embryos by treating them with ultraviolet light. The Berkeley workers first screened *Xenopus* messenger RNAs for the ability to restore the axis in the treated embryos, and then went on to clone the corresponding DNAs. They found two such cDNAs, and have so far determined the sequence of one. It proved to be none other than Xwnt-8. Smith and Harland have also shown that Xwnt-8 messenger RNA can induce a complete axis in *Xenopus* embryos, whereas activin messenger cannot.

And more recent findings have provided further evidence that activin isn't the only factor capable of inducing dorsal mesoderm. In work to be published in the January issue of the *EMBO Journal*, Christian, Moon and Daniel Olson, also in Seattle, made the surprising discovery that bFGF induces dorsal mesoderm in *Xenopus* animal hemispheres if the hemispheres are taken from embryos previously injected with Xwnt-8 messenger RNA. As mentioned before, bFGF was supposed to induce ventral mesoderm, not the dorsal portion. But independent work, in which David Kimelman and his colleagues at the University of Washington injected *Xenopus* embryos with bFGF messenger RNA, has also shown that the growth factor has dorsal mesoderm-inducing capabilities.

Those capabilities may not have been



JAN CHRISTIAN & RANDALL MOON

Two-faced. *Wnt protein injections can produce double-headed* Xenopus *embryos.*

detected in the earlier work, the researchers say, because in those experiments isolated *Xenopus* animal hemispheres were simply incubated with bFGF. That may have given a less natural result than injecting the embryos with the messenger RNA so that the embryonic cells make the bFGF themselves.

The upshot of all this? The previous model, "which I liked very much, has been thrown into some confusion by the Wnt work," Smith says. "In the extreme case, you could even throw out activin, which depresses me somewhat since I've been working on the molecule for 4 years." Smith probably doesn't need to worry, however, because the researchers generally say that activin is likely to be involved in mesoderm development, although perhaps at a later stage than previously thought. The current view is that a protein working through the Wnt signaling pathway prepares the cells at the equator of the *Xenopus* embryo and then bFGF, activin, and possibly other factors, go on to produce the Spemann organizer and other mesodermal tissues.

Both Moon and Harland sound a cautionary note, however. Although their experiments have clearly identified a signaling pathway that can be activated by Wnt-1 and Xwnt-8, leading to dorsal mesoderm induction, neither of these Wnts is likely to be the natural inducer since neither gene seems to be active early enough in the embryo. The natural inducer may be another member of the Wnt family, or a maternal Wnt in the egg, or even a non-Wnt protein.

While untangling the events leading to the formation of the Spemann organizer is important, it's only half the story. The other big question is, of course, what does the organizer do at the molecular level once it goes into action? And developmental biologists are beginning to make progress there, too.

So far, they have been focusing their attention on the homeobox genes, which are so called because they encode an evolutionarily conserved sequence of 60 amino acids known as the "homeodomain." There's a good reason for looking at these genes: A vast amount of evidence has already shown them to be important in body pattern development in species ranging from the fruit fly, *Drosophila melanogaster*, to the mouse. What's more, several research groups, including Melton's at Harvard and that of Eddy De Robertis at the University of California School of Medicine, Los Angeles, have found that some homeobox genes are turned on in *Xenopus* embryos by bFGF and activin, the same growth factors that have been linked to dorsal mesoderm induction.

Indeed, at a recent meeting* on Crete, De Robertis described new results suggesting that a homeobox gene may play a key role in organizer activity. "De Robertis has a gene that will be able to give us insight into the molecular basis of the Spemann organizer," says developmental biologist Peter Gruss of the Max Planck Institute for Biophysical Chemistry in Göttingen, Germany, who heard De Robertis talk at the meeting. (Because the paper dealing with the gene's biological activity is in press at *Cell*, De Robertis declined to comment even on the significance of the work for *Science*'s readers; however, *Science* also heard his Crete talk and the original cloning of the gene was reported in the 17 July issue of this journal.)

In that earlier work, De Robertis, Bruce Blumberg, and their colleagues set out to look for homeobox genes that are active specifically in the *Xenopus* Spemann organizer, which is located in a small bit of tissue called the "dorsal lip." The researchers succeeded in pulling out DNA clones corresponding to four such active genes. One proved to be especially interesting, partly because the protein sequence encoded by its homeobox region resembles that of two previously identified homeodomains, one from the *gooseberry* gene and one from the *bicoid* gene—as a result of which the researchers gave their gene the name *goosecoid*. The resemblance to *bicoid* was intriguing because Christiane Nüsslein-Volhard's group at the Max Planck Institute

*The meeting, "Evolution and Development: Thirty Years after the Jacob-Monod Paradigm," sponsored by the Institute of Molecular Biology and Technology in Heraklion, Greece, was held on 14 to 20 October.

for Developmental Biology in Tübingen has shown that this gene is important for head formation in the fruit fly and no vertebrate *bicoid* relative had been reported. What's more, *goosecoid* is turned on in the organizer region of the *Xenopus* embryo early in development. "What he [De Robertis] has found is a gene that very accurately marks the Spemann organizer," Melton remarks.

Those results suggested that *goosecoid* activity in the organizer might lead to axis formation, and in their more recent work, De Robertis and his colleagues tested this hypothesis by injecting *goosecoid* messenger RNA into the ventral side of *Xenopus* embryos. The result: exactly what Spemann saw when he did his transplantation experiments. "We got twinned embryos that have complete heads. We think it [*goosecoid*] establishes the axis in the animal," De Robertis said at the Crete meeting.

What's more, treatment with ultraviolet light, which suppresses axis formation in *Xenopus* embryos, suppresses *goosecoid* activity, whereas treatments that enhance axis formation also enhance the gene's expression. "We would like to conclude that this gene follows very closely the biology of the organizer," De Robertis said.

The next step is to find out more specifically how *goosecoid* exerts it effects. Since the proteins encoded by homeobox genes are generally thought to be transcription factors that regulate gene expression, the supposition is that the *goosecoid* product turns on other genes in the organizer and that their products work to bring about the formation of the head-to-tail axis.

But tracing all the genes that participate in head-to-tail axis formation won't be easy. Most researchers predict that the organizer's function, as well as its formation, will be complicated, with many homeobox genes involved. "People are just cloning these things like crazy," says Kimelman, whose own group is among those doing the cloning.

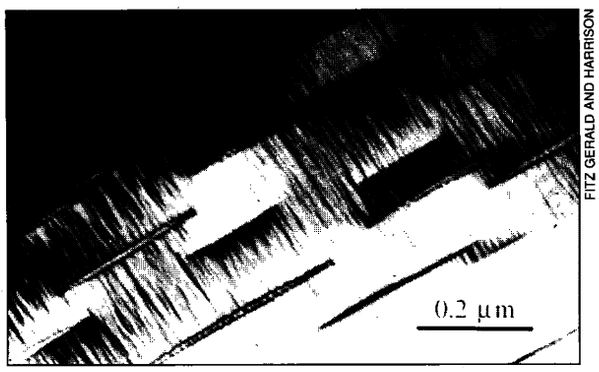
And homeobox genes won't be the only ones involved. Recently, for example, Smith's group, working with Bernhard Herrmann of the Max Planck Institute in Tübingen, identified the *Xenopus* equivalent of the mouse gene *brachyury* (meaning short tail). Genetic evidence indicates that the gene participates in mesoderm formation in the mouse, and the same now appears to be true in *Xenopus*, Slack says. It, too, is turned on very rapidly by the mesoderm-inducing growth factors activin and bFGF. What's likely to be happening in axis formation is a cascade of gene activities that starts with the Wnts and other growth factors and proceeds through the homeobox genes and their targets, until ultimately the organism's body plan is established. ■ JEAN MARX

Reading History From a Single Grain of Rock

A group of earth scientists deciphers tens of millions of years of geologic change from single specks of mineral

TO CALTECH GEOPHYSICIST OSCAR LOVERA and his colleagues, the tiny grains glittering in a chunk of crystalline rock like granite are more than inert bits of minerals—they are history books, waiting to be read. With Frank Richter of the University of Chicago and Mark Harrison of the University of California, Los Angeles, Lovera has developed a technique for reading the cooling history of a sample of rock—and, by extension, the tectonic forces that shaped it—in single, millimeters-long grains of mineral. It is "the major new thing in thermochronology," declares Peter Zeitler of Lehigh University.

Not every geophysicist is so enthusiastic, though, and that guarantees an especially lively session at a meeting of the American Geophysical Union in San Francisco this week. One topic of discussion will be the team's first demonstration of the technique: tracing the grandest tectonic process of recent geologic history, the rise of the Tibetan plateau (see box on p. 1589). Questions—some hostile—are guaranteed, since the team's findings have placed them on one side of a contentious debate about the choreography of mountain building in Asia. And even the technique itself has been encountering skepticism; critics accuse the group of minimizing its drawbacks and overstating its potential.



FITZ GERALD AND HARRISON

Pages of geologic history? *These boundaries may separate feldspar regions recording different stages of cooling.*

Lovera and his colleagues might have expected to encounter a few storms, for they have been pushing geologic dating techniques into uncharted waters. Geochronologists, as Lovera and his ilk are called, have traditionally concentrated on getting a single date from each mineral grain, a date derived from the steady, clocklike decay of some radioactive element that was locked into the mineral when it crystallized. One problem has been that the clock doesn't always start ticking at the moment the mineral grain crystallizes from magma or hot solutions, but millions of years later, when the crystal structure cools enough to trap the timekeeping element's decay product. Thus most samples actually give a single point in a drawn-out history of cooling—a history that, for a large rock mass cooling deep underground, may stretch across hundreds of millions of years.

Now Lovera, Richter, and Harrison are claiming to have found a way to read the full sweep of that cooling history. That's something researchers have long been eager to achieve, for the cooling record holds clues to the events that sculpt Earth's surface. "Virtually every important tectonic process involves discontinuities in heat flow," says Harrison. When swift erosion or faulting exposes a rock mass, its cooling rate can accelerate sharply. Sedimentation or mountain building, on the other hand, can bury a rock more deeply, slowing its cooling.

Lovera, Richter, and Harrison unveiled their single-grain technique in the December 1989 *Journal of Geophysical Research*. It builds on work that began in the 1940s, when researchers first realized that potassium-40, a radioactive isotope that decays to form the gas argon-40, could serve as a natural timekeeper in potassium-containing rocks. By extracting both potassium and argon from a rock sample, researchers could find the ratio of parent element to decay product—and thus get a measure of the rock's age. But extracting two different elements from the same sample was cumbersome, and in the 1960s workers came up with an elegant refinement: Put the rock in a reactor, where neutrons transform some of its potassium to argon-39. Then heat the rock to extract both the argon-40 decay product and the argon-39—a proxy for the parent potassium—in a single step. The ratio of the two argon isotopes would then give an age.