DEVELOPMENTAL BIOLOGY

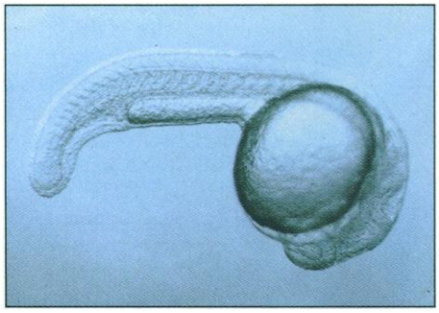
In Toulouse, the Weather—and the Science—Are Hot

TOULOUSE, FRANCE—Every 4 years, the European Developmental Biology Organization hosts an international congress to review the latest research in the field. This year's event took place here from 9 to 13 July in the midst of a heat wave. But the 800 participants from around the world willingly braved the heat to hear about recent progress on topics ranging from genes that control embryonic development to how cell fates are established and complex organs formed.

Zebrafish Yields a Bumper Crop of Genes

Who is the fastest rising star in developmental biology? If you pose this question to researchers in the field, don't be surprised if they name not a colleague, but the zebrafish. Because this common aquarium species breeds rapidly and produces beautifully transparent embryos, it's becoming a favorite of scientists trying to analyze the complex cascade of events that lead from a single fertilized egg to a complex animal. An eagerly awaited development reported at the Toulouse meeting should send its star soaring even higher.

To capitalize fully on the zebrafish's advantages, researchers need to know which genes control its development. So beginning



JUDITH EISEN/UNIV. OF OREGON

Clear winner. The transparency of the zebrafish embryo makes it easy to study.

about 3 years ago, two teams, one led by Christiane Nüsslein-Volhard at the Max Planck Institute for Developmental Biology in Tübingen, Germany, the other by Wolfgang Driever at Boston's Massachusetts General Hospital, independently embarked on large-scale "saturation screens" aimed at detecting as many mutations as possible that disrupt zebrafish development. With early results looking promising (*Science*, 13 May 1994, p. 904), it was standing-room only as Nüsslein-Volhard stepped to the podium to present the first public unveiling of the results of the now-completed Tübingen screen.

For the most part, the crowd in the packed auditorium was pleased with what it heard. "I was very impressed with the numbers of mutants they got," says Denis Duboule of the University of Geneva, who adds that the zebrafish is "the first vertebrate system which could be attacked by conventional genetics." The ultimate hope is that developmental control genes identified in this easy-to-study species might provide a steppingstone to comparable genes in other higher organisms, including mouse and man.

Over the 3 years that the project has been under way, the Tübingen group has examined approximately 1.2 million embryos created by treating adult male fish with a mutagenic chemical, mating them with normal females, then inbreeding the offspring for two more generations. The intensive screen has yielded 4000 mutants with abnormalities affecting early development of the nervous system, muscles, and numerous other organs. About 2800 were discarded, Nüsslein-Volhard says, "because they didn't seem to be special for particular development processes."

But more than 1200 were sufficiently specific to be kept for further analysis. This analysis involves mating fish with similar defects to see whether the defects are caused by the same or different genes. With that analysis about 70% complete, the Tübingen group has identified some 350 genes that are directly involved in development.

One sign of the thoroughness of the Tübingen screen is that it turned up virtually all of the handful of zebrafish genes previously identified, most of them by Charles Kimmel's lab at the University of Oregon, as playing a role in development. But the other, newly discovered genes constitute a bumper crop that should keep developmental biologists busy for years. For example, in a poster presentation, Tanya Whitfield, a postdoc in Julian Lewis's lab at Oxford University who is collaborating with the Tübingen group, described evidence for 17 genes that influence inner ear development.

Despite the general enthusiasm for the Tübingen screen, some participants in the Toulouse meeting, particularly those who study very early events in the embryo, expressed disappointment. "This was an enormous enterprise," says one researcher who asked not to be identified, "but it hasn't produced many early genes," particularly those involved in events critical to laying down the embryo's overall body plan. But Nüsslein-Volhard says that "we did not expect to find these things," adding that the so-called "maternal genes" controlling these early events—which are expressed in the egg even before it is fertilized by the sperm—are not detectable in the type of mass screen her group performed.

Nevertheless, Nüsslein-Volhard told *Science* her group has identified some early acting genes, although she says she is not ready to talk about them in public "because we are not sure about their interpretation." But she plans to tell all in a special issue of the journal *Development*, slated for later this year, that will be devoted totally to the results of the Tübingen screen. And, as the subject of its very own special issue, the lowly zebrafish will be ready for the scientific hall of fame.

Of Neural Plates and Cell Fates

"I am the master of my fate, I am the captain of my soul," wrote the 19th-century poet William Ernest Henley. But for the cells of the developing embryo, the situation is a bit more complex. Indeed, just when and how an undifferentiated precursor cell becomes "fated" to fulfill its destiny as a specialized cell in the body is one of the major unresolved questions in developmental biology. Now a presentation at the Toulouse meeting by Marianne Bronner-Fraser of the University of California, Irvine, provides surprising new information about how a group of embryonic cells, known as neural crest cells, become committed to their own particular fates.

Contrary to expectations, Bronner-Fraser's findings suggest that these cells—and perhaps other embryonic cells as well—do not become committed in a single, early step, but commit gradually over an extended period of time. According to developmental biologist Margaret Buckingham of the Pasteur Institute in Paris, Bronner-Fraser's work shows "how plastic tissues are."

Many developmental biologists had thought neural crest cells would commit early, forming a highly distinct and segregated population of cells within the tissue where they form, the neural plate. One reason for thinking so is that the ultimate fates of the neural crest cells are so different from the destinies of the cells surrounding them.

Neural crest cells arise along the outer edges of the neural plate, which in the chick begins to form about 24 hours after the egg is laid. Over the subsequent day and a half, the neural plate rolls up to form the neural tube,

the precursor of the brain and spinal cord. But unlike the neural tube cells that surround them, neural crest cells migrate throughout the embryo to form a variety of structures, including the peripheral nervous system, the cells of the adrenal medulla, pigment cells, and even some of the bone and cartilage found in the skull.

But several types of experiments led Bronner-Fraser to challenge the view that neural crest cells form a distinct population within the neural plate. When she surgically removed neural crest in the cranial region, for example, new neural crest cells began regenerating from the edges of the adjacent neural plate within a few hours—meaning, says Bronner-Fraser, "that a [cell] population that would not normally produce neural crest" can be induced to do so.

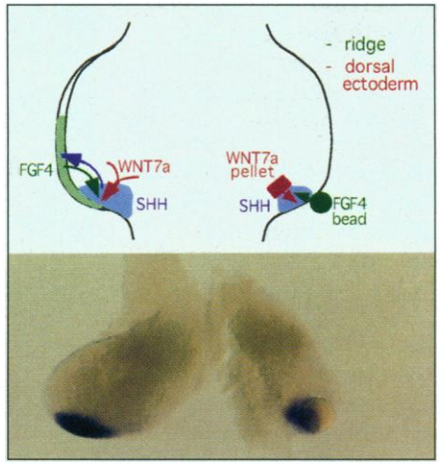
She also performed a series of grafting experiments in which she put pieces of neural plate in contact with non-neural cells of an embryonic layer called the ectoderm, which gives rise to both the neural plate and to skin cells. As a result, new neural crest cells were induced between these two pieces of tissue. Moreover, this induction could take place after the neural tube had closed, indicating that even at this relatively late stage cells that normally would go on to become other tissues—for example, brain, spinal cord, and skin—could be diverted from those fates to become neural crest. "Marianne has shown that [these cells] can do things that we never would have thought they would be able to do," says Emily Gale, who studies the neural crest in Malcolm Maden's lab at King's College in London.

These results indicate, Bronner-Fraser says, that the changes that commit neural crest cells to their fate occur gradually, rather than all at once, as they respond to the signals they receive from neighboring cells. "These cells may ... continuously experience signals that bias their fate," Bronner-Fraser says. "If they experience these signals for a prolonged time, they may then differentiate in a certain direction, for example, to become neural crest."

Bronner-Fraser is now trying to identify the genes that help determine how and when neural crest cells are formed. One possible candidate for this job is a gene called *Slug*, which is expressed preferentially in neural crest cells. In the regeneration experiments, she finds that *Slug* becomes active at the cut edge of the neural plate just before the new neural crest cells begin to form. This pattern suggests *Slug* may play a role in the signaling pathway that tells uncommitted cells to become neural crest. And a postdoc in Bronner-Fraser's lab, Meyer Barenbaum, has recently isolated a new gene that appears to be turned on in the neural plate even before *Slug* and may also be involved in some of these very early cell fate decisions. Indeed, rather than being the masters of their fate, neural crest cells may be slaves to their own and other genes.

How to Make a Limb

Last fall, a group of prominent developmental biologists surveyed by *Science* identified the molecular mechanisms underlying tissue and organ formation as one of the great unsolved riddles in the field (*Science*, 28 October 1994, p. 561). But a talk by Cheryll Tickle of University College, London, indicated that researchers may be getting closer to solving the puzzle in at least one model system: the developing limb of the chick embryo.



Y. YANG AND L. NISWANDER, *CELL* **80**, 939 (1995).

Working together. Wnt7a and FGF4 maintain Shh production *(dark stain)* in the limb bud, as can be shown *(right)* by replacing the tissues that make Wnt7a and FGF4 with FGF4-impregnated beads and a Wnt7a-expressing cell pellet. The normal situation is at left.

Tickle reviewed recent results from labs in the United Kingdom and the United States showing that three master control proteins, each acting on one of the three axes of the limb, work together to lay down the limb's three-dimensional pattern. "Rather unexpectedly," Tickle says, "it's turned out that signaling along each of these axes is mutually regulated [by] a coordinated network of signaling molecules."

As a dramatic illustration of the power of these signals, Tickle presented work from her lab showing that when an acrylic bead soaked in one of these proteins, fibroblast growth factor (FGF), is placed in the flank of an early chick embryo, it can trigger the formation of an entire new limb. (This work has also appeared in the 10 March issue of *Cell*.) "This is an incredible result," says Denis Duboule of the University of Geneva. "It means that once you initiate something ... the whole machinery starts, and you end up with a limb."

Although this startling result is a developmental biologist's dream, it comes after years of investigation into how the complex pattern of the vertebrate limb gets laid down. This work—from Tickle's lab, as well as Lee Niswander's group at the Sloan Kettering Institute in New York City, Gail Martin's team at the University of California, San Francisco, and Cliff Tabin's lab at Harvard Medical School—indicates that FGF kicks the process off when it's produced by an area of thickened tissue at the tip of the developing limb called the apical ectodermal ridge. FGF in turn induces production of the protein sonic hedgehog (Shh) by a second signaling center (the polarizing region). Shh appears to stimulate production of still more FGF, creating a "feedback loop" of gene activity that leads to continued outward growth of the limb.

While FGF controls the proximal-distal (near-to-far) axis of the limb and Shh its anterior-posterior (front-to-back) growth, a third signaling center, called the dorsal ectoderm, controls the dorsal-ventral (top-to-bottom) patterning by producing the protein Wnt7a. Like FGF, Wnt7a is required to maintain Shh expression. As further proof of the pivotal role of these signaling molecules, Niswander presented evidence at the Toulouse meeting that all three can substitute for the signaling centers that produce them.

Yet despite the dramatic progress in understanding the role of these signals, the picture of limb bud development is not yet complete, as researchers know that FGF, Shh, and Wnt7a must control other "downstream" genes to produce the complete limb structure. Tickle has found, for example, that her FGF-soaked beads induce a new wing when placed in a more anterior position on the flank and a new leg when placed more posteriorly. "Something obviously comes on later that helps to specify the differences [between wing and leg]," says Niswander.

And these downstream genes remain elusive, although some important candidates for more intermediate players have emerged. Among them are the so-called *Hox* genes, which have been found to control the patterning of body structures in both vertebrate and invertebrate embryos. And recently, Tickle and her co-workers have identified another candidate: the genes encoding a group of molecules called bone morphogenetic proteins (BMPs). Genetic studies in the chick suggest, Tickle says, that BMP lies downstream of Shh in the signaling pathway for digit formation.

But even with the unknowns about the downstream genes, the work on the chick limb bud indicates that researchers may finally be turning a corner in their quest to solve the mysteries of development. Concludes Tickle: "These experiments open up a new way to look at how the vertebrate body is established."

–Michael Balter