Looking to Development's Future

Science's survey of leading developmental biologists points to the most important questions in the field and the areas to watch for the greatest progress in the next 5 years

As developmental biology enters its second century, researchers working in the field are optimistic that they may be on the brink of solving some of its deepest mysteries. They are learning about the underlying mechanisms that form the embryo at every step from the first laying down of an organism's body plan to the formation of individual organs. The News Reports beginning on this page, along with the Perspectives and Articles that follow, examine the progress in these rapidly moving areas of developmental research.

Developmental biology had a big birthday this year—it has been a full century since the field was founded by German biologist Wilhelm Roux and his colleagues in 1894. But unlike human centenarians who are reaching the end of life, developmental biology is basking in its full-blown prime. Indeed, the excitement and promise of the field have never been greater, as researchers close in on the secrets of how a single fertilized egg cell goes through the complex and beautifully orchestrated series of changes that create an entire organism. "You could argue that developmental biology is a very ripe field," says Donald Brown of the Carnegie Institution in Baltimore. "The methods seem to be in place," he adds, for answering many of the field's biggest questions.

To commemorate the end of the field's first century, *Science* set out to find out just what those big questions are. We asked more than 100 leading developmental biologists to tell us what they thought were the most important unanswered questions in their field—and also the areas where they expect the most rapid progress in the next 5 years. Despite the wide-ranging nature of the topics encompassed by developmental biology, certain broad questions were mentioned repeatedly. And, reflecting the field's ripeness, all the key questions were in areas where respondents expect rapid progress in the next half decade (see tables on pp. 562 and 563).

Among the 66 responses, the most important unanswered question was clearly that of how the body's specialized organs and tissues are formed. What's more, this topic, known as morphogenesis, also came in second on the list of areas where rapid progress is expected in the next 5 years. Coming in second behind morphogenesis were questions about how the mechanisms of development evolved and how evolutionary processes act on development to change its outcome and generate new species. Following evolution, three topics finished in what was essentially a dead heat: how patterns form in the embryo that tell different parts what to become, how cells receive and respond to signals during development, and how individual cells become committed to particular developmental fates.

Developmental biologists are making use of all the techniques and model systems at hand to resolve these major questions, and they entertain high hopes of success—soon. "My first response would be 'rapid progress in all areas,' " wrote Maria Leptin of the University of Cologne in her response, "because there is such an enormous number of good people working in this field."

Making the organs and tissues

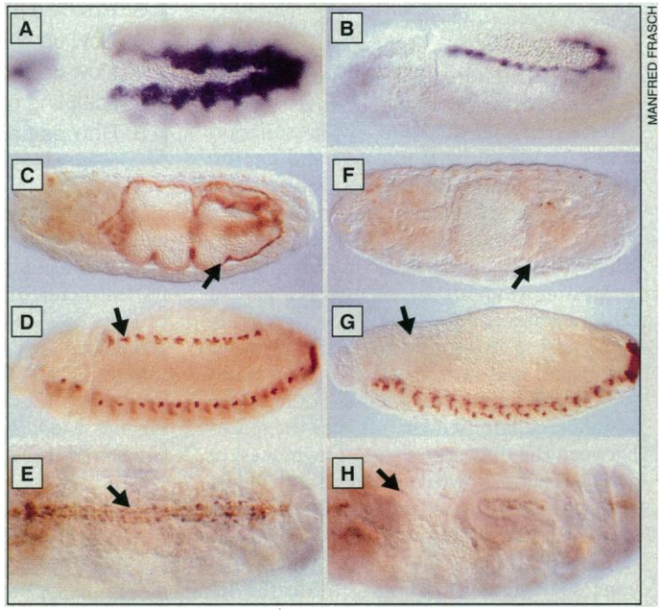
The topic that ranked highest in our survey—morphogenesis—isn't by any means the first stage of the embryo's development. Before any tissue or organ can form, earlier steps must occur, steps that tell cells who they are and what tissues they should form. If those early steps take place in the control room for development, morphogenesis, then, is what happens on the factory floor—the actual assembly of the tissues and organs that make up the plant or animal (see story on p. 564).

These days, a growing number of developmental biologists are focusing on finding the specific molecules that drive those morphogenic events. "What you really want to know is the guys that are doing the job, the workhorses," says Eric Wieschaus of Princeton University. The hope, he continues, is that knowledge of those molecules "would help you to understand the actual mechanics [of morphogenesis] from a cellular standpoint."

Morphogenesis encompasses the formation of all tissues and organs, from the first embryonic tissue layers to the finished heart, brain, or kidney. Wieschaus and Leptin are among those who are approaching morphogenesis at its earliest, and perhaps simplest stage: gastrulation, when the ball-shaped embryo folds and cells rearrange to form the three embryonic tissue layers, known as endoderm, mesoderm, and ectoderm.

Both groups are taking a genetic approach to the problem, screening many thousands of mutated fruit flies for those that are defective in genes that control gastrulation. One gene from the Wieschaus lab, called *folded gastrulation*, looks promising because it seems to control a particular kind of cell movement (a process called invagination in which cells move into the interior of the embryo) at not just one but two different times in early development. "This made us think that *folded gastrulation* is really one of the essential players in invagination," says Wieschaus.

Meanwhile, Ray Keller of the University of California (UC), Berkeley, is approaching gastrulation quite differently, manipulating frog embryos in culture to try to understand how cells apply the physical force that drives



Tinman. The blue stain shows *tinman* gene expression in (**A**) 5-hour and (**B**) 8-hour fruit fly embryos. The arrows point to (**C**) the normal gut musculature, (**D**) heart precursors, and (**E**) heart, structures all missing in embryos in which the gene is mutated (**F**,**G**, and **H**).

New Tools of the Trade

Although all the major unanswered questions in *Science*'s survey of developmental biology were also mentioned as areas where rapid progress is expected within 5 years, the converse was not true: There were several areas where rapid progress is expected that fall in the category of technical advances rather than major questions. Respondents cited two in particular: identification of new developmentally important genes, via both new methods for manipulating the mouse genome and large-scale genetic screens of the zebrafish. "The mouse gene-mapping project is likely to have profound effects on the advancement of developmental biology in all areas," responded Brigid Hogan of the Howard Hughes Medical Institute at Vanderbilt Medical School in Nashville, Tennessee. "Also, the zebrafish mutagenesis screens will become powerful tools."

One of the features adding great power to gene identification in mice is the fact that many developmentally important genes from lower animals such as fruit flies and worms have counterparts in mice. And when researchers knock out those genes in mice, they often find that the genes play remarkably similar roles in vertebrates. For example, Manfred Frasch of Mount Sinai School of Medicine in New York recently found a gene he called *tinman* that is essential for fruit fly heart development. The gene has also been found in frogs and mice, and Richard Harvey of the Walter and Eliza Hall Institute in Melbourne, Australia, recently reported that knocking out the gene in mice causes abnormal heart development.

In addition, many developmental biologists are excited about the application in zebrafish of a powerful genetic technique called "saturation screening," which allows the whole genome to be scanned for developmentally important genes. Christiane Nüsslein-Volhard and Eric Wieschaus, both then at the European Molecular Biology Laboratory in Heidelberg, Germany, pioneered the technique in fruit flies in the late 1970s. Nüsslein-Volhard, now at the Max Planck Institute for Developmental Biology in Tübingen, Germany, as well as Wolfgang Driever of Massachusetts General Hospital in Boston, is now reaping the harvest of a similar screen in zebrafish (see Perspective by Nüsslein-Volhard on p. 572).

The zebrafish screens are turning up many genes involved in organ formation, says Driever, because "the transparency of the zebrafish embryo allows us to look in the living embryo for function ... and presence of internal organs." In the heart, for example, Driever's group has found "mutations where part of the heart is missing, or one of the chambers didn't develop properly, or the valves are missing" (*Science*, 13 May, p. 904). While the full understanding of those genes is still far off, such findings secure the zebrafish, as well as the mouse, important places in the rapidly expanding toolbox of developmental biology.

–M.B.

the complex movements of gastrulation. "The community of cells does something to distort the embryo," says Keller. "The question is what does it do." By cutting out pieces of the embryo and studying the tissues in culture, he has found cell movements that account for some of the shape changes seen during gastrulation. For example, the lengthening of the long axis of the embryo is caused by cells crawling toward that axis from the sides and squeezing between each other, just as a group of people all crowding into line will force the ends of the line farther apart.

Keller plans to search in frogs for the counterparts of the fruit fly gastrulation genes turned up by Wieschaus and Leptin, then perturb those genes in frog embryos and study the resulting cell movements. "We want to go from the molecule to the cell behavior to the mechanics," Keller says.

Cell movements are an essential part of morphogenesis, but cells also need to know when to stop growing and even die, to sculpt the tissues and give them their final form. During the formation of hands and feet, for instance, cells must die to produce the spaces between the fingers and toes. The process of cell death and growth control was offered by respondents as a separate topic—ranking seventh among key questions.

As with other areas of development, advances in understanding cell death are bound to come from studies of simple systems. One example is the vas deferens in the nematode worm *Caenorhabditis elegans*. This tube, through which sperm pass into the cloaca, first develops with a closed end. The cell that blocks the end of the tube helps the vas deferens link up to the cloaca, then dies, its death creating the opening to the cloaca. Studies of this cell's death may lead to a general understanding of cell death in tube formation, says Paul Sternberg, who studies *C. elegans* development at the Howard Hughes Medical Institute (HHMI) at the California Institute of Technology.

DEVELOPMENT'S GREATEST UNSOLVED MYSTERIES

Unanswered Questions	No. of votes	Rank on other list
1. What are the molecular mechanisms of morphogenesis?	36	(2)
2. What is the connection between development and evolution?	29	(11)
3. How do cells become committed to a particular fate?	25	(4)
3. What is the role of cell-cell signaling in development?	25	(1)
5. How are patterns established in the early embryo?	24	(8)
6. How do neurons establish their specific connections?	21	(6)
7. How do cells know when to divide and when to die in the sculpting of organs and tissues?	17	(9)
8. How do transcription factors control tissue differentiation?	15	(4)

How it all evolved

The universality of tube formation is yet to be demonstrated, but Sternberg's proposal is not unduly optimistic, considering the growing number of astonishing examples of how organisms as disparate in evolutionary terms as worms and mammals use similar strategies—often the very same genes—during development (see Article by Patel on p. 581). These similarities have sparked enough interest among developmental biologists to place evolutionary subjects second on the list of major unanswered questions. Anthony Mahowald of the University of Chicago spoke for many respondents when he posed the question: "How did the multiplicity of different developmental systems evolve and yet maintain so many common genetic components?"

The answer to such questions will be found by comparing species, observing similarities and differences, and checking to see how specific genes are being exploited in each organism, according to Sean Carroll of the HHMI at the University of Wisconsin, Madison. This information may emerge naturally as researchers compare notes on different model systems, but some research-

ers are designing studies that are specifically intended to contrast the developmental schemes of closely related species.

Carroll, for example, recently examined the genes that create the detailed color patterns on the wings of butterflies. He found that butterflies utilize the same patterning genes as fruit flies, but have put them to additional uses befitting their more elaborate wings. Similar cases of genes being co-opted for novel purposes are cropping up wherever species are compared. "Evolution is opportunistic," says Carroll. "It uses what is there and modifies [it] rather than inventing things from scratch." Respondents to our survey made clear that the search to understand how evolution proceeds is an area that is going to get hotter in the future.

Laying down the patterns

Pattern formation is critical not only for the correct development of butterfly wings, but for the proper development of every part of an animal or plant. Reflecting its central role in development, pattern formation was one of three topics that essentially tied for third place on the list of key questions.

It's not that nothing is known about how patterns are formed in the developing embryo. On the contrary, the unraveling of the patterning process that divides the early embryo of the fruit fly *Drosophila melanogaster* into segments has been one of the greatest success stories of developmental biology. "We obviously don't know everything about anything, but in the early *Drosophila* embryo, we really know quite a lot," says Igor Dawid of the National Institute of Child Health and Human Development.

One thing that is known is that some of the early embryo patterns arise by maternal bequest. Work from several labs in the 1980s, for example, showed that ovarian cells of a female fruit fly place the messenger RNA from a gene called *bicoid* into what will become the head end of the egg. Bicoid protein made from that RNA diffuses through the embryo, creating a concentration gradient that is highest in the prospective head region.

When the gradient is established, bicoid acts like a master switch. Different concentrations of the protein turn on different sets of genes along the length of the embryo; those genes produce proteins that control the expression of still other genes. The set of genes turned on in each embryo region determines whether it will become a head with antennae and mouth parts, say, or a thoracic segment with wings and legs.

While the cascade of genes that sets up patterns in fruit flies is quite well understood, much of the excitement about the future cited by our respondents lies in applying what has been learned in flies to higher animals and plants—and finding the equivalent cascades that determine their body plans and patterns. Many of the so-called "homeotic genes" that determine segment identity in fruit flies, for example, have turned up in vertebrates and appear to play similar roles in the segmentation of the brain and vertebral column (see Perspective by Rubenstein *et al.* on p. 578). And this pattern applies even to plants: Comparable homeotic genes in plants control flower petal formation.

Homeotic genes in all these systems work similarly: They code for proteins called transcription factors that control gene expression. That similarity "is not pure chance," says Detlef Weigel of the Salk Institute, but shows that "controlling transcription is a fundamental thing you have to do" to set up patterns in an embryo. Indeed, the importance of transcription factors in development is reflected by the fact that understanding how they act was a topic that ranked eighth on the list of key unanswered questions and fourth among areas in which respondents expected rapid progress.

Sending the signals

Transcription factors aren't the only molecules singled out for attention by survey respondents. During the past decade, "there has been a lot of work on the transcription factors," says Mariana Wolfner of Cornell University. But, Wolfner muses, the next 5 years might well be characterized as "the half decade outside the nucleus," as more and more attention is shifted to another class of molecules: those that carry signals between cells and then interpret those signals within the cell.

Communication between cells is crucial throughout development. In the early frog embryo, for example, a protein called Noggin, discovered by Richard Harland and his colleagues at UC Berkeley, is produced by a group of cells called the organizer and causes neighboring cells to become the precursors of the nervous system. But Noggin is just one of a dozen or more proteins that seem to play signaling roles in the early embryo (see Article by Kessler and Melton on p. 596), and those roles have yet to be sorted out. "The question of just how these molecules are working, and which does what, remains a pretty big mystery," says Harland.

To resolve that mystery, developmental biologists must learn more about the biochemical cascades known as signal transduction pathways that transmit signals into the cell. Those paths are complex, but it turns out that many of the same pathways are used many times throughout development. For example, a pathway that includes a protein called Ras controls developmental tasks ranging from the making of a fruit fly eye to the formation of sex organs in the worm *C. elegans* (*Science*, 27 March 1992, p. 1640).

This repeated use of pathways in different contexts raises a question that came up over and over in responses to our survey: How do the same signal transduction pathways give rise to such different responses in different cells? "We have a limited number of receptor molecules and a limited number of cascades," says Chicago's Mahowald, "and yet cells [under the influence of these cascades] do a myriad of different things in the organism." (See story on p. 566).

Much work remains to be done to unravel these crucial signaling pathways, and the topic is not only high on the agenda for developmental biologists, but it is the area in which the most respondents expect rapid progress. "The ability to use developmental genetics to identify [signaling] pathways, combined with the ability to unravel functional relationships between pathways, will be [a] central element of the next few years of developmental biology," says Spyros Artavanis-Tsakonas of the HHMI at Yale University, who is using a genetic approach in

A DOZEN HOT AREAS FOR THE NEXT HALF DECADE

Where progress is expected	No. of votes	Rank on other list
1. Cell-to-cell signaling and signal transduction pathways	35	(3)
2. The mechanisms of morphogenesis	23	(1)
3. Vertebrate development, aided by mouse and zebrafish genetics	20	
4. Cell commitment to developmental fates	19	(3)
4. The role of transcription factors in differentiation	19	(8)
6. The identification of new developmentally important genes	17	
6. How neurons make specific connections	17	(6)
8. Establishment patterns and polarity in the early embryo	16	(5)
9. The molecular mechanisms of tissue induction	14	
9. Control of cell division and death during tissue formation	14	(7)
11. Development's relation to evolution	12	(2)
12. Plants as models for understanding development	8	

fruit flies to sort out the signaling pathway used by a protein called Notch, which plays a key role in determining cell fates in animals ranging from nematodes to humans.

How cells learn their future

One common function for signaling pathways is in determining the fate of cells. Activation of Notch in particular cells in a fruit fly, for example, makes those cells become skin cells rather than nervous system cells. The question of how cells learn their fates ranked equal to signal transduction in our survey. But a signal passed from cell to cell is not the only way cells' futures are fixed. "There is a whole separate issue, which is barely just scratched," says Chris Doe of the HHMI at the University of Illinois, Urbana-Champaign, "and that is that you can also [determine] cell fate by putting something in the cell asymmetrically."

One example cited by Doe is a protein called Numb, which is made in a particular type of cell that forms a sensory organ in fruit flies. Yuh Nung Jan and his colleagues at the HHMI at UC San Francisco recently showed that, during cell division, the Numb protein segregates at one end of the cell. As a result, one daughter cell receives all of the Numb protein; the other receives none. The daughter that gets the Numb becomes a sensory neuron, and the other becomes a support cell. "Something has to set up that first asymmetry" that sends two sibling cells to different fates, says Doe. Numb itself is a good candidate, although no one yet knows its biochemical function. As more labs focus on the nature of such cellular decisions, says Doe, a more complete picture of the role of protein partitioning is bound to emerge.

And as the respondents to our survey told us, cell fate is just one of the many topics that are ripe for the plucking as developmental biology basks in its productive prime of life. Indeed, as it moves into its eleventh decade, the field will clearly continue to rush headlong toward the answers to some of its most fundamental questions concerning how a single cell becomes a full-fledged organism.

–Marcia Barinaga

Additional Reading

W. McGinnis and M. Kuziora, "The molecular architects of body design," *Scientific American* **270**, 58 (1994).

S. B. Carroll *et al.*, "Pattern formation and eyespot determination in butterfly wings," *Science* **265**, 109 (1994).

J. Shih and R. Keller, "Patterns of cell motility in the organizer and dorsal mesoderm of *Xenopus laevis*," *Development* **116**, 915 (1992).

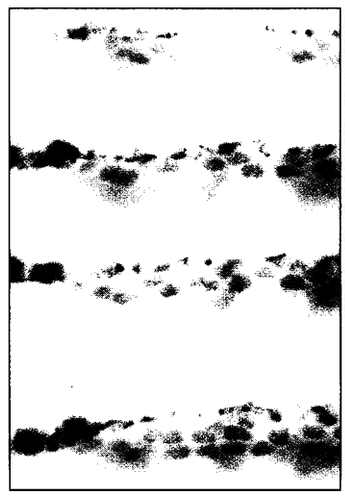
M. Costa, E. T. Wilson, E. Wieschaus, "A putative cell signal encoded by the *folded gastrulation* gene coordinates cell shape changes during *Drosophila* gastrulation," *Cell* **76**, 1075 (1994).

MORPHOGENESIS

Finding Clues About How Embryo Structures Form

When Michelangelo created his masterpiece, David, he probably used a large hammer and chisel to hew the figure's overall shape and progressively smaller tools to refine David's anatomical features from the ringlets in his mane of hair down to the cuticles of his fingers and toes. But his array of tools wasn't enormous: As the artist moved from one part of the sculpture to another, he no doubt relied on a select array of a few tools, using them over and over again to shape quite different features. Now, researchers are beginning to recognize that nature displays a comparable economy in choosing the molecular tools that shape the developing embryo.

Just how the seemingly homogeneous blob of cells that forms the early embryo is molded into the tissues and organs that form a complete living organism is a mystery that has long intrigued scientists. Even now, researchers do not yet have a complete picture of that process. One area that remains perplexing comes later in development, when organs and tissues are formed as part of the process called morphogenesis. Researchers consider understanding that process to be of the highest importance: It ranked first in *Science*'s survey of key unanswered questions in the field (see story on p. 561).



STEVE DINARDO/THE ROCKEFELLER UNIVERSITY

Together. In these four segments of a fruit fly embryo the genes *wingless* (*blue stain*) and engrailed (*brown*) are expressed in close-by cells.

Although morphogenesis is far from understood, developmental biologists are beginning to pile up clues. What they are finding is that, just as Michelangelo used and reused some of the same tools, the same families of molecules that guide the earliest stages of embryogenesis—setting up such basic elements of body pattern as the head-to-tail and dorsal-ventral axes—also help out in morphogenesis.

What is more, these molecules have been heavily conserved over the course of evolution, playing similar developmental roles in species ranging from the fruit fly to fish to mice to human beings. "Once a primordial multicellular organism figured out how to direct certain cells to become head, others to become tail, and which cells should become dorsal and which ventral, it was probably easier in evolutionary terms to elaborate on these early signaling events than to change the nature of the signals altogether," says developmental biologist Brigid Hogan of Vanderbilt University Medical School in Nashville, Tennessee.

Although there are many illustrations of this repeated reuse of developmental signals (see Article by Kessler and Melton on p. 596), one of the clearest and best understood examples comes from a family of structurally related genes that includes *wingless* (*wg*) in the fruit fly *Drosophila melanogaster* and the *Wnt* genes in vertebrates. *Wg*, the prototype of this family, was first identified by R. P. Sharma and V. L. Chopra of the Indian Agricultural Research Institute in New Delhi as the gene at fault in certain fruit fly mutants with defective wing development. Other researchers, including Nick Baker of the Medical Research Council in Cambridge, England, subsequently showed that forming wings isn't *wg*'s first appearance on the developmental stage. Far from it: Expression of *wg* also helps specify the position or organization of the 14 segments that ultimately form the major components of the adult fruit fly's body, and later helps establish the dorsal-ventral axis of the imaginal discs. "This is a prime example of a single gene being used and reused during the course of development," says Philip Ingham of the Imperial Cancer Research Fund in London, who studies development in the fruit fly and the zebrafish.

The *Wnt* genes, the vertebrate relatives of *wg*, are proving just as versatile. Roel Nusse, then a postdoc in Harold Varmus's lab at the University of California, San Francisco, identified the first *Wnt* gene, *Wnt-1*, in the early 1980s. Since then, researchers have identified at least 14 other *Wnts*, which act both early and late in embryogenesis.

Take *Wnt-3a*. Andrew McMahon's group at Harvard University has evidence from mice that this *Wnt* family member is needed for formation of the mesoderm during gastrulation, which is the complex series of cell movements that leads to formation of the three primordial tissue types in the embryo