Developmental Biology: Where Is It Going?

At a recent meeting, experts speculated on what the past means and what the future may hold for developmental biology

Ten years ago, one of the hottest areas of biological research was the molecular biology of bacteria. Then, as the mysteries of gene expression in bacteria began yielding to hordes of determined investigators, more and more of these biologists sought untapped areas of study. They turned to higher organisms and to questions in the broad field of developmental biology. But what, really, has been discovered in this past decade, and where is their new knowledge leading developmental biologists? These were the questions that speakers at a recent meeting were asked to address. The meeting was held on 11 to 13 September at the Marine Biological Laboratory in Woods Hole, Massachusetts. It was convened to honor James Ebert, the outgoing director of the laboratory, and was organized by Paul Gross, the current director of the laboratory, and by Donald Brown of the Carnegie Institution in Baltimore.

On paper, the program of 12 talks appeared to have few common threads. For example, the topics ranged from the genetics of corn to mosaic mice to fruit fly genetics. This diversity is a reflection of the enormous range of subjects generally lumped together under the heading developmental biology. At its heart, of course, the field is the study of how highly specialized tissues and organs form from a series of divisions of a single fertilized egg. In other words, as Sidney Brenner of the Medical Research Council in Cambridge, England, has said, it is the study of "how to build a mouse." Its medical arm is the study of birth defects—how and why they occur.

The question of how development occurs is so broad that it can hardly be addressed at the present time. Instead, biologists concentrate on smaller questions, such as how a few specific genes are organized and controlled and how cells recognize each other. This focus was apparent at the conference and explains why the 12 talks had such dissimilar titles. Yet from this diversity, a few common themes emerged. Underlying these was what Gross describes as "a confirmation of what everyone believes anyway"—that genes directly or indirectly regulate all that goes on in development.

One theme was that classical questions in embryology can now be rephrased in the language of today's molecular biology with its emphasis on genes. It is hoped that, in the rephrasing of these questions, insight will be gained into their solution. For example, a difficult and as yet unsolved problem is the mechanism of cell determination: What is it that commits a cell to a pathway of development that will only become apparent many generations later? How and when does a cell "know" that it will later be part of the central nervous system and not the heart? According to Gross, this question can now be rephrased as, What is it that maternal genes and genes that are active in the first few divisions of the fertilized egg contribute to the reprogramming of the genes of specific embryo cells?

Another theme was that the vast body of research on how genes act in bacteria may not carry over to gene actions in higher organisms. If the recent past is any guide, explained Howard Green of the Massachusetts Institute of Technology, future studies of the molecular biology of development are bound to be full of surprises.

The current trend toward rephrasing classical questions in developmental biology was most apparent in Gross' talk, which traced the history of theories of cell determination. Nearly a century ago, biologists found that if they separated an embryo into two parts at an early stage of its life, it would survive and develop as two normal embryos. This led them to believe that the cells in the early embryo are undetermined: each cell has the potential to develop in a variety of different ways. Later, they found that the situation was not quite so simple. It matters in which plane the embryo is cut. If it is cut in a plane different from the one used by the early investigators, it will not form two whole embryos.

A debate arose over what exactly was going on. Which embryo cells are determined, and just when do they become irreversibly committed to their fates? What are the so-called morphogenetic determinants that tell a cell what to become? But the debate could not be resolved because no one was able to ask the crucial questions in a form that could be answered. This situation changed as a result of recent discoveries in molecular biology, Gross explained. Now, investigators think they know at least some of the molecules that act as morphogenetic determinants in early development. They have been able to show that cell determination begins even before an egg is fertilized and is mediated through these substances.

Gross has found that an unfertilized sea urchin egg contains these determinants in its cytoplasm. The substances are inactive and are inhomogeneously distributed. When the egg is fertilized and divides, the substances become active and, presumably, alter gene expression. Since the substances were unevenly distributed in the egg, the two daughter cells are different from the start. Each has different amounts of these substances and so can be qualitatively different in its gene activity.

The substances that Gross studies are maternal messenger RNA's (mRNA's)—copies of certain of the mother's genes. He and others find that these mRNA's direct, in large part, the synthesis of histones, a class of proteins that bind to DNA. Once synthesized, the histones move to the cell nucleus and sections of DNA wrap around them to form a structure that resembles beads on a string. The beads are the DNA segments wrapped around histones; the string is the intervening DNA.

Gross describes the process of cell determination as self-generating, starting with the first cell divisions. There is an enormous burst of histone synthesis during early development, and this burst is correlated with a decrease in the distance between the beads of DNA and histones on the DNA string. "Contrary to prior beliefs that genes become active during development, it now looks like genes become progressively inactive," he says. In other words, the driving force behind development is not the increasing

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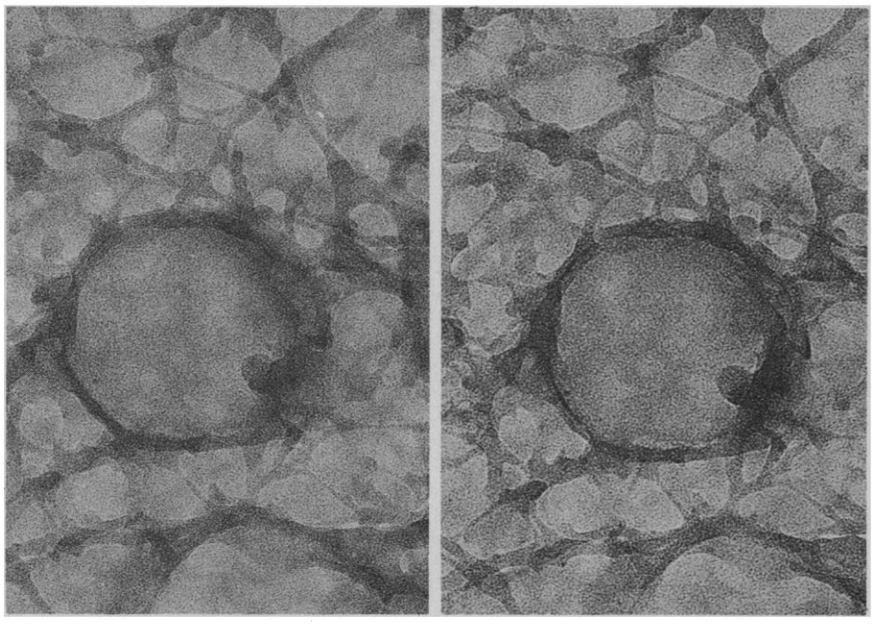

Stereo pair of electron micrographs showing the fine structure of the cytoplasm of a normal rat kidney cell. The cytoplasm appears as a lattice of slender strands ranging in diameter from 50 to 100 angstroms (× 100,000). [Source: Keith R. Porter, University of Colorado]

activity of genes but their selective inactivity.

If Gross' scenario is correct, it is important to know how gene-regulating substances come to be inhomogeneously distributed in egg cells. This problem was addressed in a talk by John Gurdon of the Medical Research Council in Cambridge, who suggested that the answer might come from so-called injection experiments. The idea is to take purified molecules, inject them into cells, and see whether the molecules know where to go in the cells and, if so, determine how they know.

Gurdon finds that if he removes nuclear proteins or certain cytoplasmic proteins from egg cells and then reinjects them, they find their way back to the right part of the cells. "There is no reason why you can't modify proteins and see what portions of them are necessary to find their way back to the right spots," he said. With this knowledge, researchers can propose and test mechanisms that might explain how these portions guide the proteins to their appropriate positions in cells.

The idea that proteins and mRNA's have specific positions in cells seems odd when one considers the cell cytoplasm to be fluid. But Keith Porter of the University of Colorado argued in his talk that cytoplasms are more gel-like than fluid. Porter looks at cells with high-voltage electron microscopy, which allows him to see three-dimensional pictures. What he finds is that the cytoplasm seems to have a lattice structure that changes in response to temperature and chemical conditions of the environment. The lattice consists of microfilaments, which are structures containing contractile proteins, and microtubules, which are tube-like protein structures involved in changing cell shapes and in cell division.

The theme that the molecular biology of higher organisms may be different from that of bacteria was touched on often during the conference. But it was the major focus of Brown's final talk.

Brown predicted that when biologists finally come to understand how genes are controlled in higher organisms, they will discover that there is no single mechanism. Already, he said, a diverse group of mechanisms have been found that alter genes and thus may be involved in controlling them. Brown explained that "one of the features of these mechanisms of gene alteration is that they are used only by higher organisms. That is the prevailing theme—the remarkable difference between mechanisms of higher organisms and those of bacteria."

Among the ways in which higher organisms, but not bacteria, alter genes are elimination (the germ cells of the worm *Ascaris* eliminate 70 percent of their chromosomes, for example), amplification (in amphibians, there are thousands of copies of ribosomal RNA genes), specific chemical modifications, and heterochromatization, which Brown described as "the glubbing together of certain chromosome regions." (He pointed out, however, that there is as yet no evidence that DNA regions thus massed together are inactive.)

Also unlike bacterial mechanisms are the ways in which cells of higher organisms may modify gene expression by altering RNA copies of the genes. Portions of the RNA's are cut out, Brown explained; the RNA's are also chemically modified and they are somehow stabilized so that they can remain quiescent in cells until needed.

As an example of the kinds of surprises he has had when working with genes from higher organisms, Brown described some recent studies of what he says are the simplest of these genes—those that encode 5*S* ribosomal RNA. He purified the genes, attached them to a plasmid, and allowed them to be transcribed in a test tube. Then he asked, What portions of these genes contain the start and stop signals for transcription? In bacteria, a start signal is at one end of each gene and a stop signal is at the other.

Systematically, Brown cut into the gene from its beginning, expecting, by analogy with bacteria, to cut out the start signal for transcription. "But we found the 5*S* RNA genes do not work that way. We had to delete more than one-third of the gene before we saw a stopping of accurate initiation." Then Brown started deleting sequences from the other end of the gene, expecting to find a termination signal immediately. Once again, he had to delete a large portion of the gene to destroy accurate termination. What he found, then, is that an interior region of the gene, rather than flanking regions, controls accurate initiation and termination of transcription.

As for the future, Brown predicted that "we will soon be able to decipher the DNA part of gene controls. We should be able to alter the controls of genes and then see what controls the controls." It will be harder, Brown confessed, to decide how cell proteins alter gene expression. But he does not think the difficulties are insurmountable. What all this means to developmental biology, Brown said, is that in the next 5 years biologists should arrive at a molecular explanation of cell determination.

Brown's prediction seemed quite optimistic to some, especially considering that the study of higher organisms has led to so many unexpected findings. Yet as biologists face the fact that the secrets of development of higher organisms may not yield so easily—that the fruit fly is not just a "flying coli," as Walter Gehring of the University of Basel put it—they are also coming to realize that they already know how to address some of the major questions in development. And that is the first step toward answering them.—GINA BARI KOLATA