The Riddle of Development

Studies of the genes of frogs suggest that some very "mundane" biophysical principles may explain how development works generally

The riddle of development is in essence the riddle of gene control. Explaining how an embryo develops in an orderly fashion from a single fertilized egg cell to an organism consisting of many different cell types requires an understanding of the ways in which specific genes are turned on or off to give the cells their final constellation of traits and then of how those traits are maintained.

One of the best-studied and best-understood gene control systems is that of the 5*S* ribosomal RNA genes of the frog *Xenopus laevis*. Donald Brown, whose laboratory at the Department of Embryology of the Carnegie Institution of Washington (which is in Baltimore) has been at the forefront of that research, recently talked with *Science* about the lessons learned from the 5*S* RNA gene system and what they might reveal about development. "It struck us that there are some potential generalities for how development works," he says.

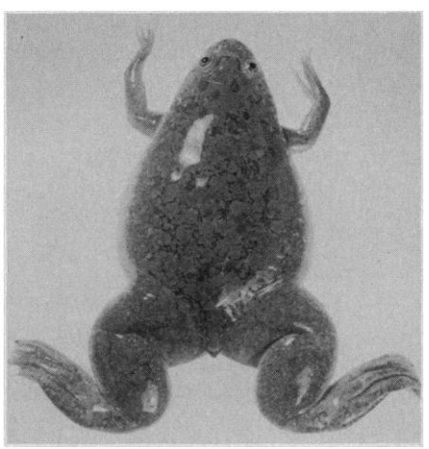
The 5*S* ribosomal RNA genes encode the smallest of the three major RNA's that are needed to make the ribosomes, the small particles on which proteins are synthesized. A haploid *Xenopus* cell contains some 20,000 of the genes, which are subdivided into two families. Members of the larger family, which contains about 98 percent of the total number, are transcribed into 5*S* RNA only in egg cells and are thus referred to as the oocyte 5*S* RNA genes.

The smaller family includes about 400 genes, or 2 percent of the total. These differ slightly in structure from the oocyte genes and are transcribed both in egg cells and in all the somatic cells of *Xenopus*; they are called somatic 5*S* RNA genes. The question then is, Brown says, "Why are the oocyte genes turned on in oocytes and off in somatic cells?"

About 5 years ago, researchers began to get their first clues to the way in which gene activity is controlled in higher organisms. For example, they learned that there are specific regulatory sequences in the DNA that are necessary for gene transcription. In a somewhat surprising development, Brown and his colleagues showed that the control region for 5*S* gene transcription lies in the middle of the gene, within the coding sequence. The comparable control sites for most other genes are located just before the start of the genes.

Meanwhile, Robert Roeder, who is now at Rockefeller University but was then working at Washington University School of Medicine, and his colleagues were finding that transcription of the 5*S* ribosomal RNA genes requires at least three protein factors in addition to the transcribing enzyme, which is called RNA polymerase III (pol III). They isolated one of these, which they designated TFIIIA. This factor has a molecular weight of 40,000 and binds to the same DNA sequence that the Carnegie workers had identified as the control region for the 5*S* RNA genes.

The other two factors interact in some way with the 40-kilodalton (kD) protein and the gene to form an active transcription complex. Pol III then recognizes this complex and transcribes the gene, but the enzyme is not itself a part of the complex. The oocyte 5*S* RNA genes require the same three transcription factors and polymerase as the somatic 5*S* RNA genes.



An egg-laden Xenopus *female. [Source: Richard Grill, Carnegie Institution of Washington]*

One of the most noteworthy characteristics of the transcription complex, according to Brown, is its great stability. "We can show that many, many rounds of RNA can be made on a gene without dissociating the transcription complex." The great stability is the result of the cooperative nature of the binding. Work from Brown's and Roeder's groups indicates that the binding of the whole complex is much tighter than that of the individual factors. "It is clear that not only are there protein-DNA interactions here, but that there must also be very specific protein-protein interactions," Brown explains.

To determine what accounts for the repression of the oocyte 5*S* RNA genes in somatic cells, the Carnegie workers used somatic cell chromatin to study transcription of the two types of genes. (Chromatin is the natural complex of chromosomal DNA and its associated proteins.) As long as they treated the chromatin gently, the somatic 5*S* RNA genes were transcribed with just the addition of pol III. But the oocyte genes, even when supplied with all three transcription factors as well as the polymerase, did not work. Something was blocking their activity.

That something turned out to be one of the chromatin proteins, the histone H1, according to Mark Schlissel of the Carnegie group. When the histone was removed, the oocyte 5*S* RNA genes became accessible to the transcription factors, although all three factors and the polymerase had to be added for transcription to take place. The results showed that as long as the 5*S* RNA genes were bound to histone H1, they were not accessible to transcription factors, and conversely that genes already in active transcription complexes were not repressed by histone H1 concentrations that could prevent the formation of the active complex.

Chromatin DNA has a structure resembling a string of beads. At periodic intervals the DNA strand wraps itself around a core of histone proteins to form the bead portions, which are called nucleosomes. The histone H1 is not a part of the nucleosome core. When it is absent the core proteins can slide along the DNA. But when H1 is present, it apparently anchors the nucleosomes to the DNA, which may be how it helps block the access of transcription factors to the 5*S* RNA genes.

"All this says something about the state of oocyte and somatic genes in somatic cells. Gene activity is specific in the sense that it requires gene-specific [transcription] factors. These factors are not associated with the repressed oocyte 5*S* RNA genes in somatic cells. Repres-

sion of the oocyte 5S RNA genes involves general repressors because the nucleus is full of nucleosomes and H1," Brown concludes. "Both these states are stable, but they are caused and maintained by different molecules. The active transcription complex is stable because of the cooperative binding of these multiple factors to the promoter of the gene, the internal control region in the 5S RNA genes. The repressed state is stable because nucleosomes are anchored by histone H1, and that somehow keeps even an excess of transcription factors from getting into their cognate control region."

The activated state appears to be the more stable of the two, however, at least in the test tube. "It looks as if it would be much easier to turn on a repressed gene than to inactivate an active one."

A system in which genes become stably activated or repressed by binding appropriate proteins may greatly simplify the everyday life of the transcribing enzymes of higher organisms. In addition to pol III, these include RNA polymerase I (pol I), which transcribes the genes for the two larger ribosomal RNA's, and RNA polymerase II (pol II), which works on the protein-coding genes. There are indications from a number of investigators that pol I and II may also recognize complexes of DNA with their own transcription factors. In contrast, the RNA polymerase of bacteria generally recognizes specific DNA sequences.

Because eukaryotic cells have roughly 1000 times as much DNA as bacteria, their polymerases would have a major logistic problem if they had to pick out the right genes to transcribe just on the basis of relatively short DNA segments. But, as Brown points out, the specificity of pol III for genes that have bound the correct set of transcription factors coupled with the enzyme's inability to recognize DNA that is locked in nucleosomes by histone H1 could largely solve the problem. "This very nicely makes 90-plus percent of the eukaryotic DNA invisible to the polymerase." Similar considerations may also apply to pol I and II.

Results such as those obtained with the 5S ribosomal RNA gene system may help explain how a mature, differentiated cell can maintain its characteristic constellation of active and repressed genes for long periods of time. These cells divide infrequently, if at all, and genes in stable active or repressed complexes would be subject to little perturbation. Even if a component of the active transcription complex should be damaged, perhaps nicked by one of the endogenous protein-splitting enzymes, it could be easily replaced. "The cooperative influences permit a certain amount of turnover, as long as one or more components of the transcription complex remains localized at the control region and there are free molecules [of transcription factors] in the cytoplasm," Brown notes. "The results suggest it takes much higher concentrations of molecules to form the complex than to maintain it."

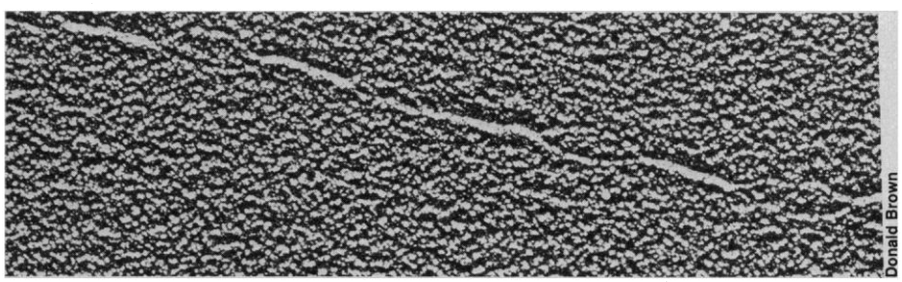
The next developmental feature addressed by Brown, "taking a rather long jump," is cell determination, in which an embryonic cell becomes committed to a particular fate, to become a liver or blood cell, for example, and not a muscle cell. Commitment requires the specific activation of some genes and the inactivation of others. The course that a particular gene will follow, Brown proposes, depends on such basic biophysical properties as the relative concentrations of the required transcription factors compared to the concentrations of molecules that might inactivate the gene, and the gene's affinities for all those molecules. It is these factors that will determine whether a transcription complex will form or whether the gene will be repressed. Once the decision is made the resulting state is stable.

In the case of the 5S ribosomal RNA genes, the concentration of the 40-kD transcription factor correlates with the developmental behavior of the genes. The factor concentration is very high in oocytes, where there are about 1 million copies per 5S RNA gene and both the oocyte and somatic genes are on. As the cells of the embryo divide, the concentration declines progressively until there is only about one copy for every four 5S RNA genes. In addition, in test tube assays the somatic genes have an affinity for the 40-kD factor that is about four times that of the oocyte genes.

These results imply that in somatic cells the somatic 5S RNA genes can outcompete the oocyte type for the very limited amount of factor. Consequently the oocyte genes, which are not protected by the transcription complex, become stably repressed by binding histone H1. Although the fourfold greater affinity of the somatic genes for the 40-kD factor is by no means sufficient to account for the difference in the expression of the two types of 5S genes in somatic cells, the Carnegie group has preliminary evidence that the test tube assay of the binding constants may not fully reflect the situation in the living cell.

Commitment of a gene by formation of a transcription complex does not necessarily mean that it will be expressed. Embryonic cells may become committed to a particular fate long before they begin to show the appropriate characteristics. The transcribing polymerase must be available and additional factors, such as hormones, may also be required to activate the gene. Brown defines differentiation as the expression of a committed gene under the influence of these additional modulating factors. He cites as examples the ovalbumin genes of the avian oviduct and the yolk protein genes of liver. Each type of tissue is committed to express the appropriate genes but does not do so until they come under the influence of the steroid hormone estrogen. The hormone in combination with its receptor may directly or indirectly change a stable but nonfunctional transcription complex into one that can be recognized by pol II, Brown postulates.

If stable transcription complexes play the role in commitment that Brown proposes, then the complexes must be capable of being transmitted to the daughter cells during cell division. This could present a problem. "Cell division is the one time in the life of the transcription complex when it must be perturbed," Brown notes. "When the [replication] fork goes through, the complex must either be split up or distributed intact to one of the two daughter genes." Nevertheless, as long as at least one of the multiple factors remains bound to each of the duplicated



5S ribosomal RNA genes.

In Xenopus *DNA, the genes are arranged in tandem repeating arrays, shown here as bars of double-stranded DNA separated by loops of denatured single-stranded DNA. Each gene occupies about one-third of the double-stranded segment.*

genes and the daughter cells contain free factor molecules, the cooperative nature of the binding should help to ensure that complete complexes will again form around the two genes. If the entire complex stays with one of the genes, a stem cell lineage results.

One of the most difficult challenges faced by embryologists is how to account for asymmetric cell divisions during development. Often when an embryonic cell divides the daughters are not equivalent. They become committed to different fates, which can even include cell death.

The basis for the asymmetric divisions is apparently established in the egg cell. Embryologists have long observed that many egg components are asymmetrically distributed in the egg and may be unequally inherited by the daughter cells when the fertilized egg divides and during subsequent embryonic cell divisions. The unequally distributed components presumably contain the molecules that determine cell fates. Brown suggests that the determinants may be transcription factors. Asymmetric divisions, he notes, "must begin in early embryogenesis because the determinants, the transcription factors, are asymmetrically localized in cells and are thus unequally distributed to the daughter cells." In that event, when the transcription complex is disturbed during DNA replication, it may reform around the gene in one daughter cell, but not in the other. Without the complex the second gene is then prone to inactivation. If the genes activated by the transcription factors include those that themselves encode transcription factors, a cascade of changes can be produced.

A great deal more work will be required to test these models of development. As Brown puts it, "We can model all these states, but that is trivial. What is not trivial is how to prove it."

One of the first requirements is to show that pol I and II work the same way as pol III, that they, too, recognize stable transcription complexes containing multiple components. As already mentioned, there are indications that this may be the case. Such demonstrations are especially critical for pol II because this enzyme transcribes the protein-coding genes. One cell type is distinguished from another mainly by the proteins they produce. The ultimate proof that the various polymerases act on transcription complexes would be the duplication of the gene control systems in the test tube, but that will require the isolation and purification of the necessary factors.

Brown is optimistic that the models he is proposing will at least prove to be useful guides to further experimentation even if they are not borne out in detail. "In my view, developmental control of genes is going to boil down to some very mundane biophysical principles," he predicts. "It's going to involve the concentrations of activator versus repressor molecules. It's going to involve cooperative influences between molecules and binding constants of proteins to each other and to DNA. I think these things can be tested with modern methods."

—JEAN L. MARX

Additional Reading

1. D. D. Brown, *Cell* **37**, 359 (1984).
2. M. S. Schlissel and D. D. Brown, *ibid.* **37**, 903 (1984).

Acid Rain's Effects on People Assessed

Acids in the air may harm lungs, acids in water may mobilize toxic metals, but it is too soon to assess risks

The effects of acid rain on some lakes, rivers, and streams have been the subject of many studies, largely because of the sensitivity of fish and other aquatic organisms to acidification. The effects on crops, forests, wetlands, soils, and buildings have also been thoroughly studied. The potential for adverse effects on human health, however, has not received comparable attention. "The ecological toxicologists have simply not been human-oriented," says Robert Goyer of the National Institute of Environmental Health Sciences (NIEHS).

The available evidence about direct and indirect effects of acid rain on human health remains inconclusive. But, according to a 1983 report from the House Committee on Appropriations, "we have learned from other environmental problems that events in the plant and animal world can serve as sentinels for the human population. Prudence dictates that we heed these warnings in the case of acid rain." The committee thus requested that NIEHS and the Environmental Protection Agency assess present knowledge about such health effects. One outcome of this request was a recent "Conference on Health Effects of Acid Precipitation" at NIEHS.*

If the results presented at that conference were to be summarized in one word, that word would still be "inconclusive." There is suggestive evidence that breathing the trace quantities of sulfuric and nitric acids formed in the atmosphere from power plant and smelter emissions is injurious to human lungs. It is difficult to extrapolate results obtained with animal studies to effects on human lungs, however, and even more difficult to separate the effects of acids from those of other air pollutants in epidemiological studies. The acidification of water supplies leads to increases in the concentration of certain potentially toxic metals in that water, but those increases have not been linked to health effects. "About the only thing we can say with any confidence," says Goyer, "is that there appear to be no serious effects resulting from contact of acid rain with the skin."

"At this point in our studies," says Morton Lippmann of the New York University Medical Center, "I think it is clear that we cannot adequately describe the nature and the extent of the effects of the inhalation of acidic pollutants on human health. We just don't know enough about either population exposures or exposure-response relationships to make a satisfactory risk assessment. We do, however, know a great deal about some aspects of the problem. We know, for example, that acidic air pollutants have created health problems in the past."

The best example of such effects was reported earlier this year by the late Tetsuzo Kitagawa. He studied some 600 cases of severe lung disease that occurred over a period of 8 years in a small part of the city of Yokkaichi in Central Japan. All of the victims lived relatively close to a titanium dioxide pigment plant that emitted 100 to 300 tons of sulfuric

*Held 15 to 16 November 1984 at the National Institute of Environmental Health Sciences, Research Triangle Park, N.C.