EARLY DEVELOPMENT IN ANIMAL CELLS

by Albert Tyler

A Caltech biologist and his associates add some pertinent information to our knowledge of early embryonic development

Our study of developmental biology at Caltech is concerned primarily with the activation of the egg—the way in which a chain of events is set into motion that transforms the egg into an adult. The problems on which we concentrate deal with the "turning on" of the synthetic processes that take place during early embryonic development, when new substances—particularly new proteins—are made for the new individual.

The work of molecular biologists during the last decade has revealed the basic steps that any cell employs in synthesizing new proteins: DNA (deoxyribonucleic acid), containing all the hereditary material, makes an RNA (ribonucleic acid) that has all of the information that the DNA has but in inverted form. This complementary RNA then serves as the template to make the protein.

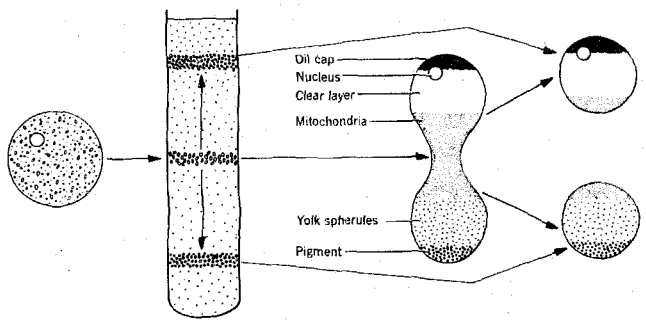
There are many different DNA's in a cell, each capable of specifying a different protein. The DNA's are always found in the nucleus, though not exclusively. In the nucleus of any particular cell, most of the DNA is inactive—not doing anything except replicating when the cell divides. But a portion of the DNA in any particular cell—let's say the nearly mature red blood cell—is active, producing the messenger RNA to make the specific proteins (principally hemoglobin in this case) characteristic of that cell. This messenger RNA goes out of the nucleus into the cytoplasm, associates with some particles there called ribosomes, and forms a structure called a polysome (polyribosome). The various amino acids are then assembled on that structure to produce the new protein.

In most species of animals the ripe, unfertilized egg is a resting cell. It is not engaged in much manufacturing activity. Immediately after fertilization, however, active protein synthesis begins. How does this come about? Is there a signal given to the nucleus to uncork some of its DNA so that messenger RNA—and new proteins—will be produced? Or is the messenger RNA already there, and are the ribosomes inactive and unable to be assembled into the polysomes?

We explore these problems in various ways. For example, we have examined the polysomes and ribosomes of sea urchin eggs: We separate them by centrifugation in tubes containing a solution of some viscous material, like sucrose, in various concentrations. Depending on whether these particles are single or grouped together (as they must be when protein is being synthesized), they will sediment at different rates and can be collected separately. When we explore these smashed-up cells, we find that before fertilization the cell has ribosomes mostly in single form, and after fertilization many ribosomes are joined together as polysomes. They are joined by messenger RNA.

This is one kind of experiment we use to study the turning on of protein synthesis in fertilization. We also know that the machinery in the *un*fertilized egg is quite capable of synthesizing protein. This has been shown with cell-free, protein-synthesizing systems that we prepare in the laboratory from homogenized sea urchin eggs. When we provide such systems with a particular set of instructions for manufacturing proteins—in the form of a simple synthetic ribonucleic acid, such as polyuridylic acid—a very simple protein, polyphenylalanine, is produced; and this occurs as actively in the systems prepared from unfertilized eggs as those from fertilized eggs.

One conclusion drawn early from this work (later shown to be erroneous) was that the unfertilized egg is inactive because it does not have messenger RNA, and that upon fertilization the nucleus produces the necessary messenger RNA. To examine this proposition, we prepared non-nucleate fragments. This is done by placing sea urchin eggs in a



In studies of early development, the centrifugation method is used to produce large quantities of non-nucleate fragments from eggs. Eggs are subjected to a centrifugal force of about 10,000 times gravity, causing them to pinch apart into two sections—one with a nucleus, one without—that can be examined for their ability to manufacture new protein.

centrifuge tube with a sucrose solution of increasing density from top to bottom and subjecting the eggs to a centrifugal force of about 10,000 times gravity for about 15 minutes. The eggs then stretch out, because their light contents go up and their heavy contents go down. They become dumbbell-shaped and pinch apart into two fragments, one of which has a nucleus and one of which does not. The two kinds of fragments form separate layers, which are collected separately and explored as to their ability to manufacture new proteins when development is initiated by artificial means.

The results of these experiments show that, even without the nucleus, the fragments can synthesize new proteins—and do it just as well as the nuclear fragments or the whole egg. The non-nucleate fragments contain the instructions for early development, although in an inactive, or masked, form. It is masked messenger RNA that is the subject of much of the present exploration in our laboratories.

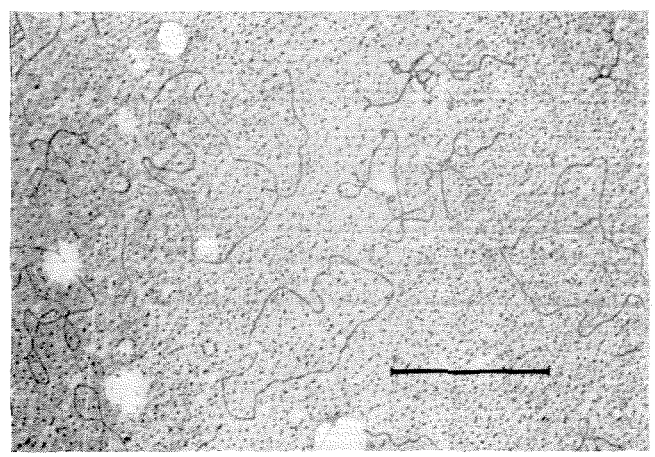
The inference that there is a masked messenger RNA is based on the supposition that there is not any other DNA outside the nucleus that might be present in the fragment and that might be activated upon fertilization. However, we know that there *is* other DNA outside the nucleus. At the same time we know that, upon fertilization, this DNA does not get immediately activated; it is not responsible for the bulk of the proteins that are synthesized in early development.

The evidence is supported, too, by the fact that the antibiotic dactinomycin, which inhibits DNA-primed RNA synthesis, permits protein synthesis and early development to proceed normally. Thus we find that the instructions (in RNA's) for early development are almost all present in the unfertilized egg. It also appears now, from work in various laboratories throughout the world, that the production of inactive messenger RNA is a common process at all stages of development. Thus the developing embryo anticipates events that are to occur at a later time by producing the working blueprints in masked form.

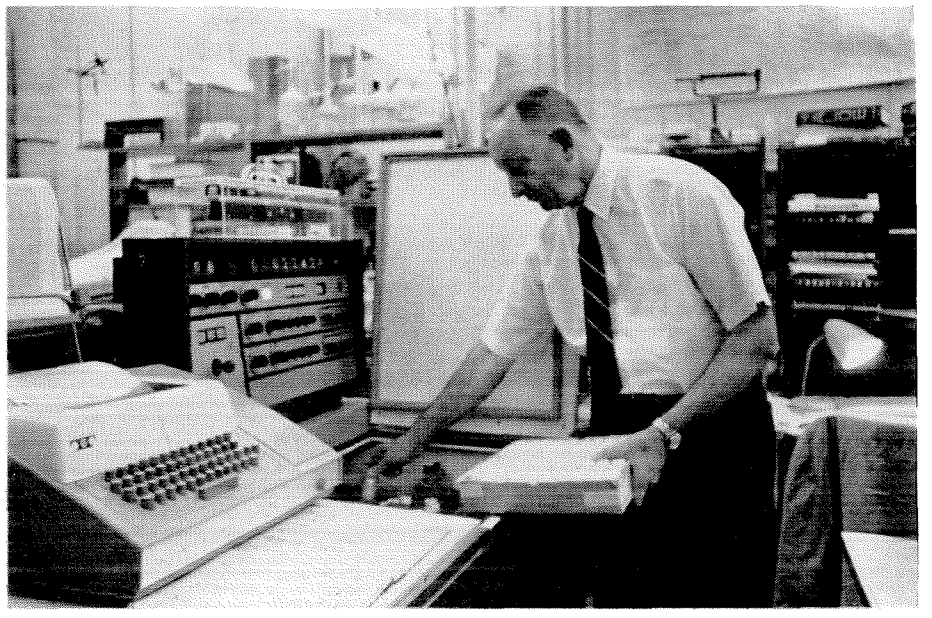
The DNA outside the nucleus is of great interest in itself. In recent years investigations with various kinds of animal and plant cells have shown that DNA occurs in the minute, rod-like particles called mitochondria that contain many of the cell's enzymes. My colleague Lajos Piko (now chief of the developmental biology laboratory at the Veteran's Administration Hospital in Sepulveda, Calif.) and I have been studying the cytoplasmic DNA of sea urchin eggs for some time.

There are about 200,000 mitochondria in a sea urchin egg. Probably the human egg-cell has about the same amount. The DNA of the mitochondria is somewhat different in density from the DNA of the nucleus. We can separate the two by the method of buoyant density centrifugation, on Cesium Chloride gradients, developed primarily by Jerome Vinograd, Caltech professor of chemistry and biology.

About a year and a half ago, researchers in Amsterdam found the mitochondrial DNA of mouse and chicken cells to be in the form of circular molecules about 4.5 microns in perimeter, instead of the long strands of nuclear DNA. At about the same time Dr. Piko and I found the mitochondrial DNA of sea urchin eggs to be in the form of circles of approximately the same size. Other workers have since found such circular DNA of similar size in other kinds of cells. We have shown also that this DNA can function as effectively as can nuclear DNA for the synthesis of RNA in an *in vitro* system. Our analyses indicate that there are on the average



Electron micrograph of DNA from the mitochondria of the sea urchin egg. (The line represents one micron.) Mitochondrial DNA occurs in the form of double-stranded circles of close to 4.5 microns in perimeter.



Albert Tyler, Caltech professor of biology, places vials containing radioactively labeled proteins and nucleic acids into a scintillation counter to measure the amount of synthesis that has taken place in a particular experiment.

about 1.3 circles of DNA per mitochondrion. Since no partial circles are found, we conclude that most mitochondria contain only a single circle of DNA, and some may have more than one.

Circular DNA was originally discovered at Caltech by Robert Sinsheimer, professor of biophysics, in a bacterial virus. Subsequently, Dr. Vinograd and his co-workers found it in the polyoma virus and other tumor viruses. The present information indicates that closed circular DNA is a normal feature of the cytoplasm of many, and probably all, organisms. In fact, measurements have shown the circles to be mostly of similar size in many kinds of organisms. Not all of the circular DNA, however, is in the form of the 4.5 micron circles. Recently Dr. Vinograd and his colleagues found a proportion in the form of double-size circles and many in the form of interlinked circles of standard size forming chains of two, three, four, or seven. These were found in HeLa cells (cultured cells derived from a human tumor) and in lymphocytes from leukemic patients. We have also found these double-size and catenated forms in sea urchin eggs, so we can assume that they are not simply a feature of tumor or other abnormal cells.

It appears, then, that the mitochondria have their own genetic machinery, although their precise contribution to development and heredity is still largely unknown. Because DNA is sensitive to mutations, mitochondria could be involved in various losses of functional capacity such as occur in the aging process. This could be true if mutations occurred that altered or inactivated the functions of these submicroscopic energy-releasing entities. For the problems of the start of development we think that the exploration of this cytoplasmic informational material may provide us sometime with some further clues as to the nature of the controlling influences. We already know that a large supply of mitochondrial DNA is a general situation for the start of development in all animals, and the relative amount of cytoplasmic DNA decreases as development proceeds. It also appears that the mitochondria manufacture proteins that move out into the surrounding cytoplasm, and that they in turn may accumulate certain proteins made in the surrounding cytoplasm.

The studies going on in developmental biology in many laboratories throughout the world have given us some preliminary insight into the factors that control early development. On the more practical side, these studies may be expected some day to lead toward more effective methods of both quantitative and qualitative control of reproduction and development. Society has become increasingly aware of the importance of quantitative control to more readily limit family size to that which is manageable, and to prevent populations from disastrously outgrowing resources. For qualitative control, the methods that have been considered until recently have been those of eugenics (namely selective breeding), which are objectionable to many people. Now, however, such control can be envisaged by non-eugenic methods, such as the use of instructional RNA's at the various stages of development, presumably with the greatest effects at the start of development. In such ways we can hope to produce better offspring—better in the sense that they are better able to cope with the exigencies of the environment, stronger, more resistant to disease, brainier, and better able to get along with one another peacefully.