Studies on Fertilization and Early Development

Caltech biologists, working on the general problem of fertilization, have obtained new information on how the development of a new living creature is initiated.

Dr. Albert Tyler, professor of biology, and some members of his team – Drs. Lajos Piko and Hector Timourian, research fellows; and Paul C. Denny and Joram Piatigorsky, graduate students – are currently trying to discover what turns on protein synthesis at the start of development. Their research, done mostly with sea-urchins and other marine animals which produce large numbers of eggs, is supported by the Public Health Service and the National Science Foundation.

Synthetic activity is very low in the unfertilized egg, although it has all the machinery for the accelerated manufacture of the proteins that are the building blocks of life. Until recently, it was thought that the sperm, in fertilizing the egg, triggered the DNA's in the egg's nucleus to produce messenger RNA's – the DNA's serving as templates for the production of the RNA's, and the RNA's in turn serving as the templates on which the proteins are made. Messenger RNA's play key roles in cell differentiation; they determine which protein – hair, heart muscle, fingernail, or whatever – a cell will manufacture.

Dr. Tyler's group has now shown that messenger RNA's for proteins that are formed during early development already exist in the unfertilized cell.

In one experiment, the Caltech biologists spun unfertilized sea-urchin eggs in a centrifuge until most of the cells were pulled into two sections. One section included the nucleus, containing the DNA; the other section had no nucleus. The cell sections with no nucleus were then treated briefly with a solution of butyric acid. This chemical is one of many that can cause an entire egg to start developing and synthesizing protein as though it were a fertilized egg. In this experiment, the cell section with no nucleus shows this same activity, synthesizing protein for several hours until, apparently, its messenger RNA's wear out.

The results are the same whether the measurements are made on the intact egg, segments of the egg, or a cytoplasmic homogenate (cell-free system).

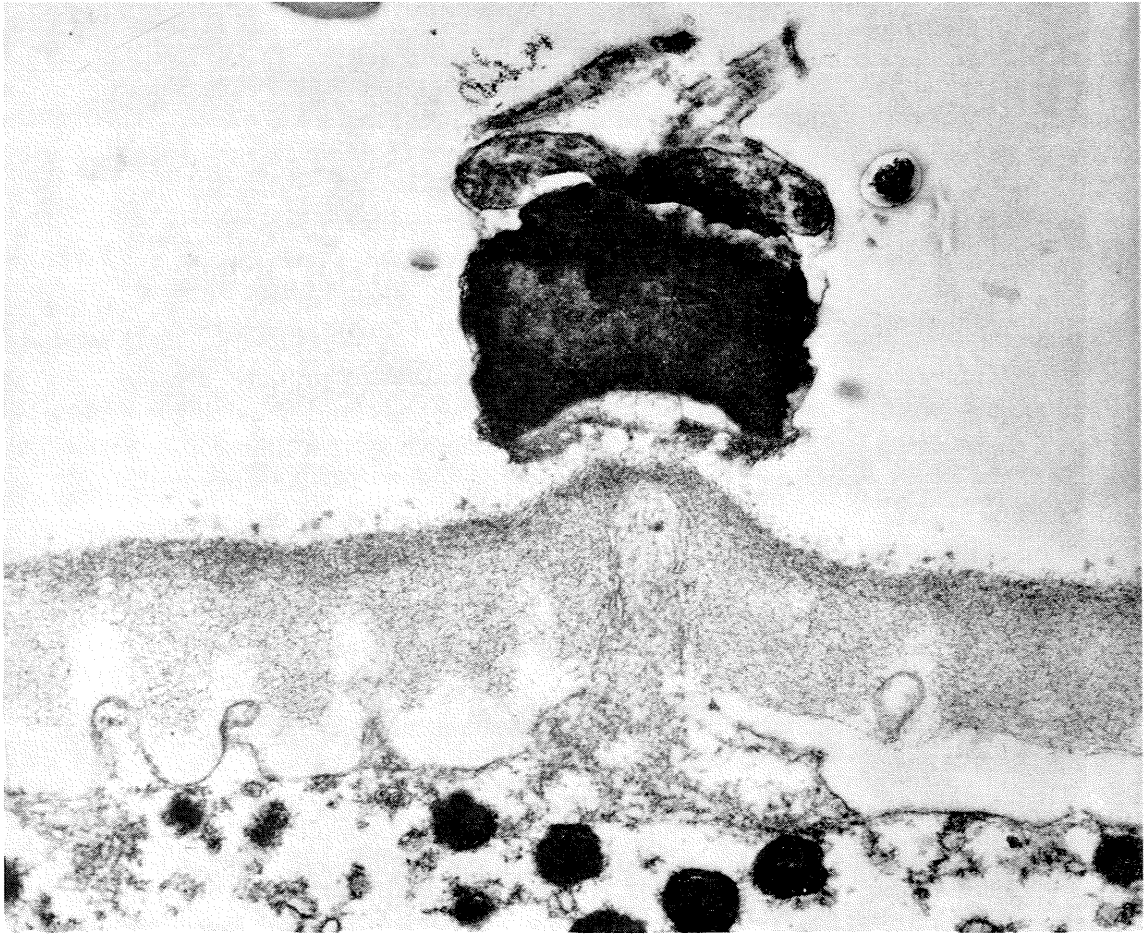
Cell-free systems that synthesize proteins are prepared by disrupting the cells and removing most of the "debris." The active system then consists of small particles of about 250 Angstroms called ribosomes, amino-acid-activating enzymes, "transfer" RNA, and extra energy sources.

Such systems manufacture the type of protein specified by the messenger RNA's that would ordinarily be present in the cells. After the endogenous messenger RNA's have "worn out" in the homogenates, the system will respond to new messenger RNA's – including even synthetic ones such as polyuridylic acid – and will manufacture the corresponding protein. For example, polyuridylic acid specifies formation of polyphenylalanine.

Cell-free systems from unfertilized eggs have very low activity. However, when polyuridylic acid is given to them, they respond quite as actively as do those developing from eggs. This shows that excess protein-synthesizing machinery is present in the unfertilized egg and ready to go to work. Messenger RNA's for the manufacture of proteins of the early embryo are present too, but are masked. Fertilization (or artificial activation of the egg) unmasks the natural messenger RNA's. Certain ions, particularly magnesium and potassium, may be part of the controlling mechanism.

A former Caltech research fellow, Professor A. Rich of MIT, using different systems, has shown that the actual protein-synthesizing site is a cluster of ribosomes (polysomes) tied by a strand of messenger RNA. The Caltech group has demonstrated that, upon fertilization, polysomes form concomitantly with the increase in protein synthesis. The polysomes can be separated and the messenger RNA liberated by lowering magnesium-ion concentration. Experiments along this line, now in progress, open new possibilities for the analysis of cell differentiation.

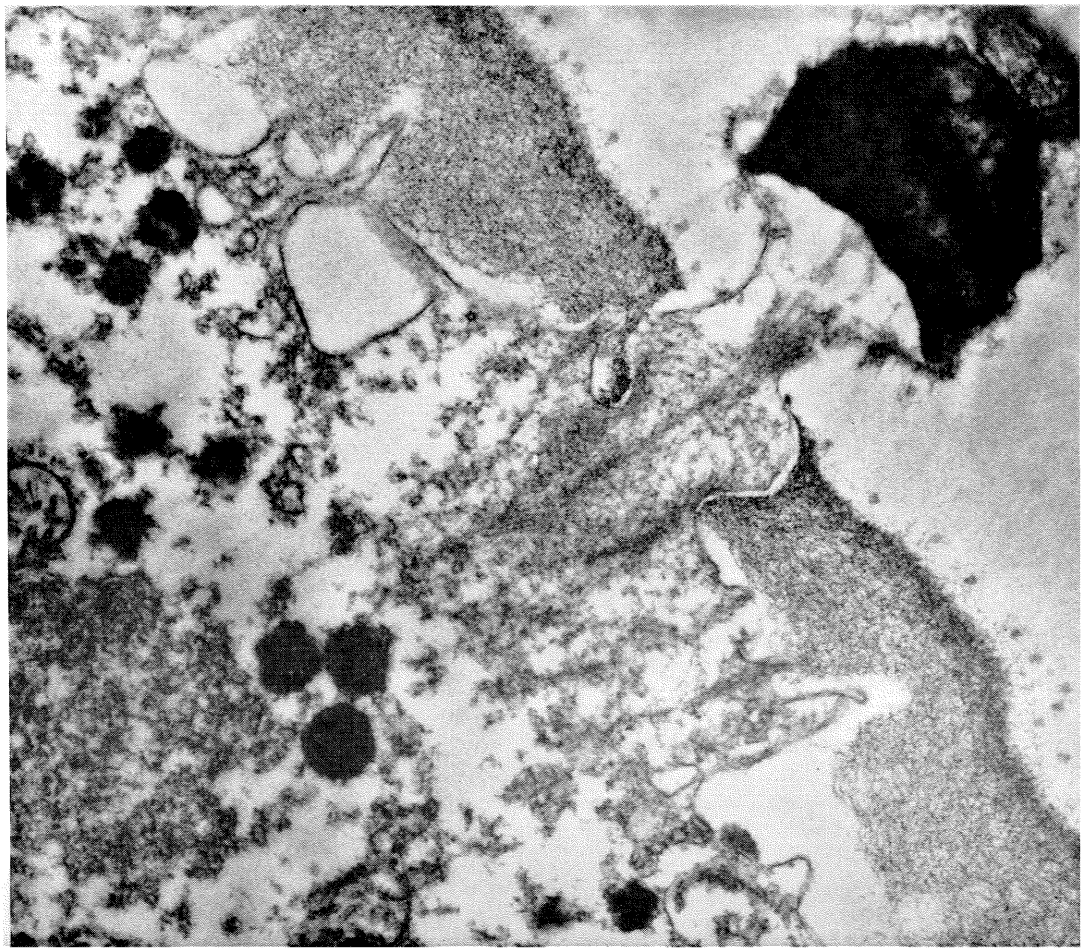
The Caltech researchers have also found that polyuridylic acid can activate the *intact,* unfertilized egg so that it incorporates various amino acids into protein. This has abnormal effects on development. However, one can envisage the possibility that, by manipulations with messenger RNA's, development may someday be controlled in beneficial ways.



ONE MINUTE
AFTER
INSEMINATION

The sperm's surface membrane unites with the egg's plasma membrane, which protrudes in the form of finger-like processes (microvilli) through an overlying coat (vitelline) membrane. Magnified 27,000 times.

THE ENTRANCE OF A SPERM INTO AN ANIMAL EGG

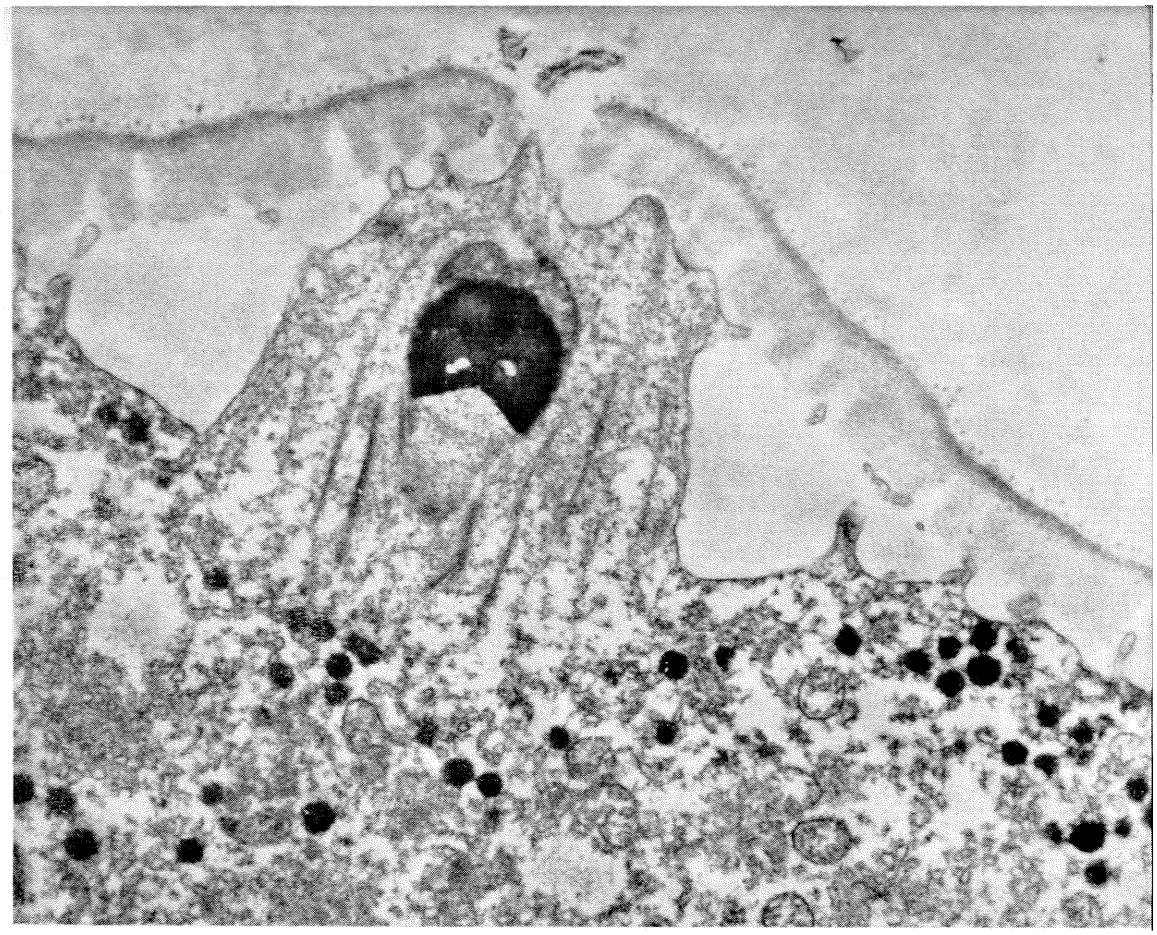


THREE MINUTES
AFTER
INSEMINATION

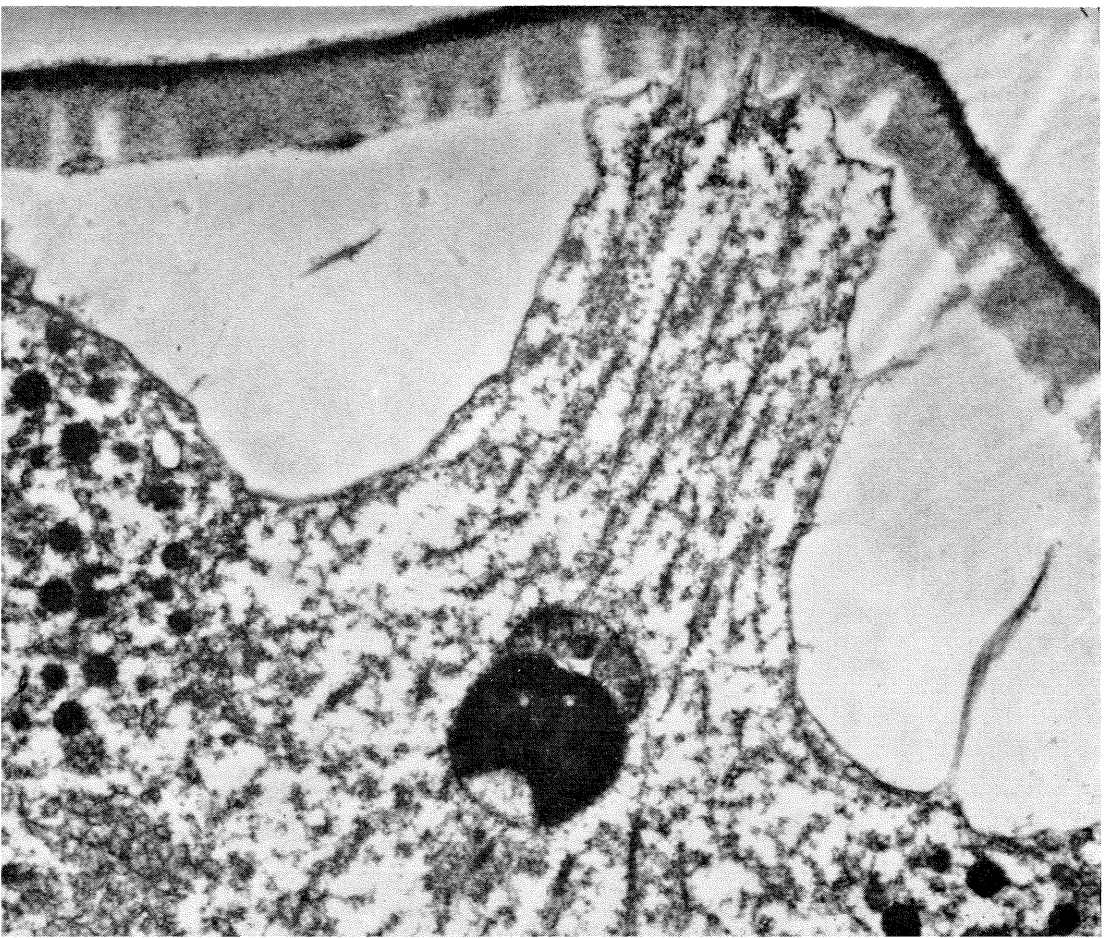
As they join, the two membranes break down so that the sperm nucleus becomes exposed to the egg-cytoplasm that flows toward it. Magnified 27,000 times.

FIVE MINUTES
AFTER
INSEMINATION

The nucleus of the sperm is squeezed through the opening in the vitelline membrane but gets through the egg surface, or plasma membrane, without there being any openings to admit it or any holes left after its entry. Magnified 13,000 times.



THESE remarkable pictures, taken at Caltech with the electron microscope, are thin sections of the sperm and egg of a sea worm known as *Urechis caupo*. The sections are about 500 Angstroms thick. The diameter of the sperm head is 1.8 microns. The egg, only partly shown, has a diameter of 100 microns.



SEVEN MINUTES
AFTER
INSEMINATION

The nucleus of the sperm is drawn further into the egg, which has formed an entrance cone with structural elements that are probably concerned with the further progress of the sperm towards the place where it meets the egg nucleus. Magnified 13,000 times.