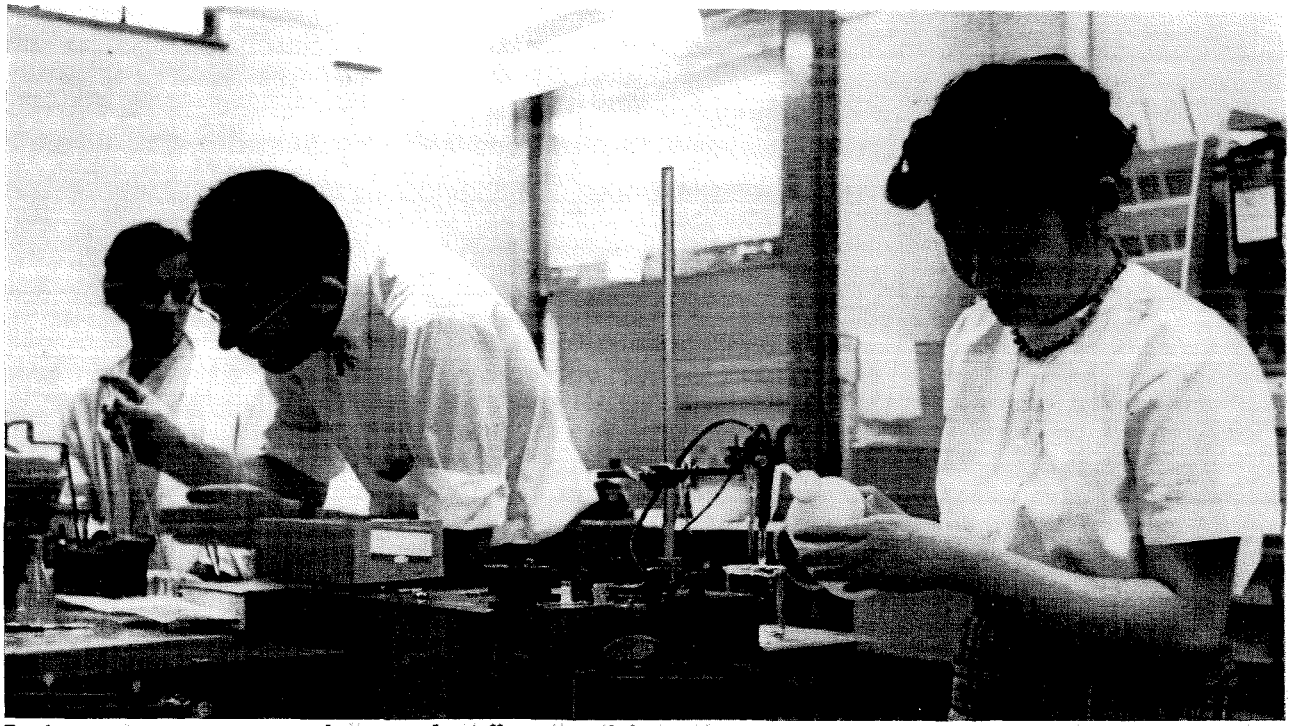

Professor James Bonner and Research Fellow Ru-Chih C. Huang prepare genetic material for histone studies.

Histone — A Protein Key?

by Graham Berry

Caltech biologists have discovered what may be the "protein key" that locks and unlocks the activity of the genes which regulate the processes of life.

The discovery provides a tool for the study of the basic life process by which two cells in the same organism are able to specialize – one, for instance, becoming a heart-muscle cell and the other a bit of eye retina. This tool or key is a simple protein called histone.

Dr. James Bonner, professor of biology, and Dr. Ru-Chih C. Huang, research fellow in biology, have succeeded in showing in research with fast-growing pea embryo cells that when histone "sits" on genes, it inactivates them – presumably turning off their particular protein-making activities – and that when the histone is removed, genetic activity is turned on again.

Genes carry the blueprints that enable nature to duplicate new generations of animals and plants. Every one of the millions of cells that make up a human being contains a complete blueprint of that individual, consisting of perhaps one hundred thousand genes, grouped in chains called chromosomes. A portion of the blueprint is for making the protein structures found in every cell and another portion is for making specialized proteins such as hemoglobin, brain, and skin.

It is the activity of these particular parts of the blueprint, and the way in which their genes transmit instructions to the cell's microscopic chemical factories, that are intriguing to Drs. Bonner and Huang. Their research is supported by the U.S. Public Health Service.

Is every gene in every cell active all the time? If this were true, then all the cells would try to produce hemoglobin, fingernail, and the scores of other kinds of proteins that the body manufactures. They couldn't do it, of course, and there would be no such complex organism as a human being – which, because of its many specialized cells, is capable of doing a great variety of complex things, like thinking.

Presumably only a few selected genes are turned on in any one cell, depending upon its specialized task; the rest of its genes must be turned off, according to Dr. Bonner. It may be that during the life of a particular cell, certain of its genes may be turned on and off several times.

Bonner and his group have evidence that histone is

the key that turns the genes off, and that the absence of histone means that they are turned on. It has been known for some time that histone is associated with the genes in humans, animals, and plants, but its significance has been a mystery.

Histone is a small, simple protein molecule composed of about 150 amino acid building blocks. There are 20 different kinds of these blocks and they are assembled into proteins by being strung together in chains. Histone is said to be simple because it is composed principally of only two of the 20 amino acids, lysine and alanine. Indications are that 80 percent of the genes in pea cell embryos are covered with histone and only 20 percent are not.

Dr. Bonner and his group selected pea embryo cells as research aids because they are very active, multiply rapidly, and are easily obtainable. Since the basic processes of living cells – whether in peas or people – are similar, and since human cells as well as pea cells contain histone, it is most likely that histone's role is general among living cells.

To obtain quantities of the genetic material, the Bonner group has developed a clever "pea-popping" technique that chops embryo pea stems and slits open the walls of their cells to yield quantities of intact cell nuclei containing the genes linked in chromosome chains. The chains are made of DNA (deoxyribose nucleic acid), which comes in long, fibrous strands coiled in a helix. Parts of the DNA strands of the chromosome are covered with histone.

Indications are that the DNA chains transmit their instructions in these three steps: 1. Those genes sending out instructions synthesize bits of another kind of nucleic acid, RNA (ribose nucleic acid). 2. The strips of RNA are complementary copies of the DNA genes. Probably for structural strength, each RNA strip is placed on a strip of nucleoprotein, the RNA-protein combination being called a ribosome. 3. The ribosome moves out of the cell's nucleus into the main body, or cytoplasm, of the cell. Here the ribosome assembles amino acids into an enzyme which, in turn, participates in the conversion of the cell's food into building blocks for making more cells.

The Bonner group is concerned with the first step in this process – the transfer of a coded message from DNA to RNA. Obtaining their raw DNA material from the "pea popper" machine, they tested the ability of this material to synthesize RNA. In precise laboratory experiments, they found that some factor in the new DNA was suppressing this synthesis. Considering the possibility that the factor might be histone, they chemically removed the histone from the DNA. Then found that the RNA-synthesizing ability of the DNA had increased five-fold.

Dr. Bonner interpreted the five-fold increase in activity to indicate that the presence of histone had inactivated four of five genes, and that when the histone was removed, the four inactivated genes became active and synthesized RNA copies of themselves, just as the fifth gene had been doing all the time.

Next, the researchers mixed active DNA with histone and the DNA stopped making RNA. When they separated histone from the DNA, the DNA activity was restored.

"In the laboratory we have separated DNA and histone and thereby increased the synthesis of messenger RNA," Dr. Bonner explains. "We have added histone and observed the drop in RNA synthesis as histone and DNA unite. And we have separated the two again and noted the increase in RNA synthesis. This last procedure is evidence that the histone does not harm the DNA and that possibly some genes may be turned on and off more than once during the cell's life."

Binding histone to DNA stabilizes the DNA structure, the researchers discovered. This was shown by the fact that the melting temperature of histone-DNA is several degrees higher than that of pure DNA. Microphotographs show that when histone becomes fastened to the DNA, the DNA strands get twice as wide. It has been suggested by others that the two strands of DNA may become harder to separate if histone is present.

"We know qualitatively that there are a dozen or more different kinds of histone," Dr. Bonner says. "We are sure that nature has had good reasons to use these different kinds and we are studying this now."

The findings shed light on the question as to why some cells have a lot of histone and some have only a little. For example, the unfertilized egg of an animal has a great deal of histone. This suggests that the genes of the unfertilized eggs are "turned off," as might be expected. Human cancer cells have less than the normal amount of histone. This could mean that too many of its genes are "turned on," which could account for a distinguishing feature of cancer cells – the apparent loss of their ability to specialize.

The discovery raises a host of interesting questions: What is it that tells the histone which genes to inactivate? How does the mechanism, whatever it is, know when to do this? What mechanism removes histone from a gene – if this happens? Does a specific kind of histone bind with a specific kind of DNA?

The Bonner group is investigating these questions. Also, they want to know which way the histone-inactivation system goes – if it goes in one direction. They want to know whether the embryo starts out with most of the genes in its cells turned off and histone somehow is selectively removed from the genes as the organism grows older. Or are most of the genes turned on in the embryo's cells in the early stages and then selectively turned off as time goes on?

In looking for answers to questions such as these, the biologists are moving into the field of cell differentiation, and a consideration of how cells begin to become specialized cells.