Summer 1980

Gene Control and Enzymatic Zippers: [Dr. Vincent G. Allfrey]

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THE ROCKEFELLER UNIVERSITY

RESEARCH PROFILES

SUMMER 1980

"We have entered the cell, the mansion of our birth, and started the inventory of our acquired wealth."

ALBERT CLAUDE, 1974

This is the first issue of a new quarterly publication from The Rockefeller University. As its name implies, each issue will profile an area of research at The Rockefeller, not only to report what's new and what looks promising, but also to help convey something of the process of science.

Vincent Allfrey and his colleagues, whose work is described in this issue, comprise one of more than 60 laboratory groups at work on the University's 15-acre campus in New York City. They are heirs to a 79-year tradition defined by the University's motto, pro bono humani generis*—for the good of humankind.*

When the University began, as The Rockefeller Institute for Medical Research, it was the only such institution in the country. It remains today one of the world's leading centers for biomedical science, having added along the way the country's first hospital dedicated solely to clinical research, distinguished faculties in the physical, mathematical, and behaviorial sciences, and a graduate education program that, in its brief 25-year span, has produced two Nobel Prize winners.

All scientific enterprise begins as a quest for deeper understanding of the natural world. Implicit is the hope that the knowledge gained will indeed contribute to human good. Or, as Dr. Allfrey puts it: "In basic research you always have the conviction that what you do is going to have a practical outcome. The question is, Where?"

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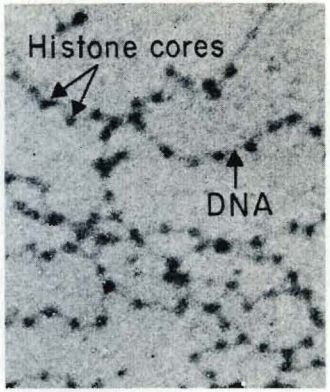
Gene Control and Enzymatic Zippers

"With regard to the process by which a cell becomes transformed to a tumor, I have always been convinced that studies of gene control are at the heart of it. That is, to really understand how cancer happens you have to understand gene control. It may be, of course, that tumors will eventually be handled by some immunological or other process and that our more basic approaches will not turn out to be that urgent clinically. But I still think it's important to know why a tumor develops, what the initial events are—whether it's simply DNA damage or an upset of the control mechanism of the cell. And since we do find that the control mechanisms are altered in tumor cells, that's the aspect of cancer that fits into the interests of my laboratory."

Professor Vincent G. Allfrey, quoted above, is a cell biologist who has spent more than 30 of his 58 years at The Rockefeller University, most of them peering and probing into the nucleus of the living cell. Specifically, his research centers on the role of nuclear proteins in the control of gene structure and function. His work began as an attempt to understand the mechanisms of gene control. Now, many years later, some



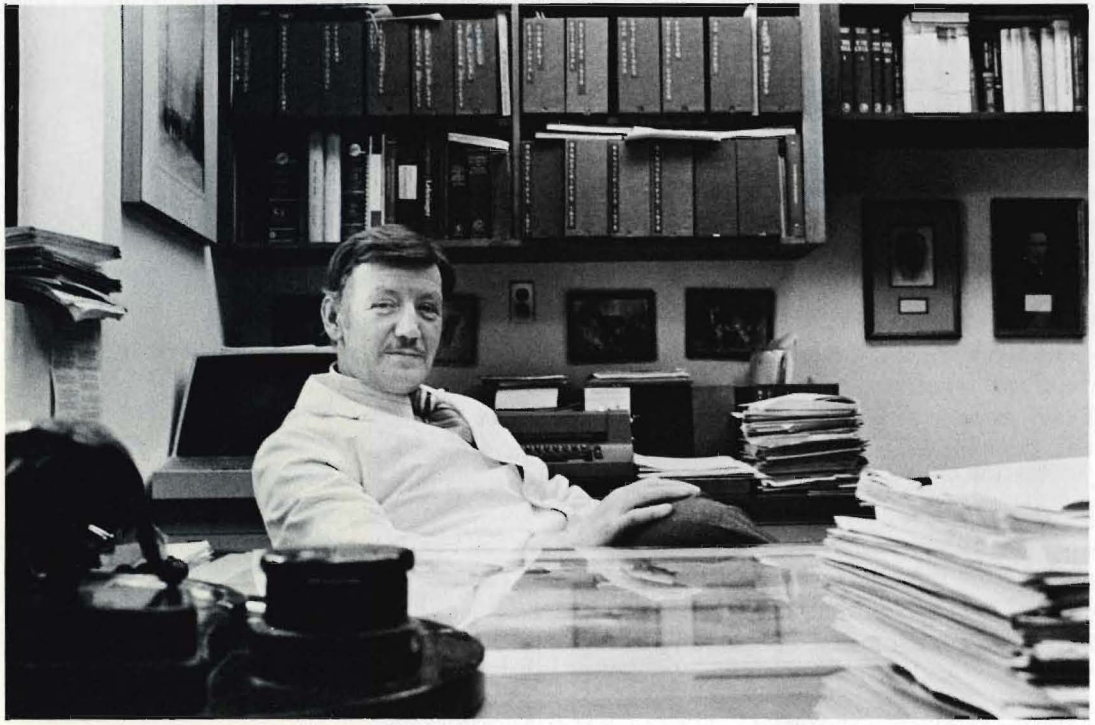
Electron-micrograph shows chromatin (magnified 100,000 times) in inactive state. DNA strands are tightly wrapped around histone "cores" (nucleosomes), each of which contains eight histone molecules. (Drawing on page 4 shows possible ways in which nucleosomes are altered when genes are activated.)

lines of this research are beginning to relate to cancer processes: studies of changes in the nuclear proteins of cells undergoing transformation to the malignant state in response to chemical carcinogens, and new approaches to cancer chemotherapy based on the inhibition of protein synthesis.

WHAT SWITCHES GENES ON?

As Dr. Allfrey explains: "The activity of every living cell is determined by its genetic material, deoxyribonucleic acid—DNA. In higher organisms, the DNA is concentrated inside the cell nucleus, in association with a number of proteins, on threadlike structures called chromosomes. Every cell contains within its chromosomes all the information necessary for the manufacture of every protein in the entire organism. Proteins are the principal functional constituents and structural elements of living matter.

Dr. Allfrey



"But no cell uses all that information. It uses only a small fraction, perhaps ten percent. Even so, it may be making 10,000 different proteins. However, many of the 10,000 proteins expressed in a liver cell are specialized, different from the proteins in a brain cell, while many are common proteins, needed for basic housekeeping functions. If you look at a pancreas cell and a red blood cell, you find that one is making digestive enzymes and the other hemoglobin. Each cell has the same information in its nucleus. Each could be doing what the other does, but it doesn't. What determines what information gets used? What switches on certain genes in the DNA? What is the difference in the chromosomes of those two cells? That's what we want to find out."

For the information in the genes to be activated, or, in the language of the geneticist, expressed, the genes' chemical code must be encoded in another chemical, a form of ribonucleic acid called mRNA, the m standing for messenger. Each mRNA carries a coded message to the ribosomes, the organelles in the cell where the message is "read" and the synthesis of specific proteins takes place. The process of message-copying from the DNA template is catalyzed by other kinds of proteins called RNA polymerases, enzymes which assemble the RNA chain from smaller subunits. If the DNA is not accessible to the polymerases, the DNA is essentially nonfunctional.

If stretched out to its full length, the DNA in a single human cell would form a thin fiber about a yard long. But the cell has to store all this material in a very tiny space within the nucleus—only a few 10,000ths of an inch in diameter. To achieve this compaction, the DNA coils around clusters of

small proteins called histones, which are predominantly found in 'core' particles that contain eight histone molecules. The DNA makes two wraps around a cluster, moves on to the next cluster, and so forth.

"There is a lot of pretty solid evidence," Dr. Allfrey notes, "from work done in our lab and in other labs over the last 20 or 25 years, that histones and other DNA-binding proteins control structure and influence the activity of the genes. Ultimately, we hope to determine which proteins control which genes. We apply our experiments to a variety of systems: normal cells during embryonic differentiation, cells responding to hormones, and cells responding to carcinogens."

THE APPRENTICE YEARS

Like the University itself, Dr. Allfrey's lab, on the eighth floor of the Tower Building, attracts scientists from all over the world; but Dr. Allfrey is a native New Yorker, born not very far from the University's campus on the east side of Manhattan. His interest in chemistry was nurtured at Stuyvesant High School, which for generations has attracted New York youngsters enthusiastic about science. He was still in his teens when he took his first job at The Rockefeller, as a lab helper, to help put himself through night classes at City College. After college and military service in World War II, he spent another year at the University as a technician before going to graduate school.

The years of his apprenticeship coincided with some of the most significant events in modern cell research, a good number of which happened at The Rockefeller. Albert Claude, George Palade, Keith Porter, and a succession of gifted colleagues developed techniques for using the electron microscope to study the deep structure of cells, beyond the reach of the light microscope. Almost concurrently, they introduced the cell fractionation technique for simultaneously breaking up billions of cells and isolating for analysis the tiny organelles revealed by this powerful new tool. The first electron micrograph of this new world within the cell was made by Claude, Porter, and Edward Fullam in 1944. Two years later, Claude published two papers summarizing a decade of work on fractionation of mammalian cells by use of the high-speed centrifuge.

Looking back on those revolutionary discoveries and related advances by scientists at The Rockefeller University and elsewhere that produced methods for analyzing cellular components chemically, Dr. Allfrey observes: "What they did was bring it all together—a combination of microscopy, cell fractionation, and biochemical techniques—so you could make a concerted attack on such very important problems as how proteins get made and secreted."

The same year the first electron micrograph was taken, three other scientists at The Rockefeller, Oswald T. Avery, Colin MacLeod, and Maclyn McCarty, identified DNA as the material of the genes—the blueprint of life—an event that has since been described as the greatest discovery of biological science in this century.

The DNA story provides a wonderful example of the unpredictable ways of science. Avery and his co-workers were not, strictly speaking, geneticists or cell biologists. They were bacteriologists at the Rockefeller Hospital, trying to come up with a treatment or preventive vaccine for pneumonia. They did come up with a serum treatment, the best that was available until the advent of antibiotics. But they also found that DNA from one strain of the pneumonia bacterium could enter and transform another strain conferring upon it and its progeny the capacity to make a new type of cell surface. In other words, the DNA was carrying genetic information from one bacterial strain to another. (Among his duties, the young lab helper, Vincent Allfrey, purified batches of pneumococcal DNA for Avery's studies.)

During this period, also, Alfred Mirsky first isolated chro-

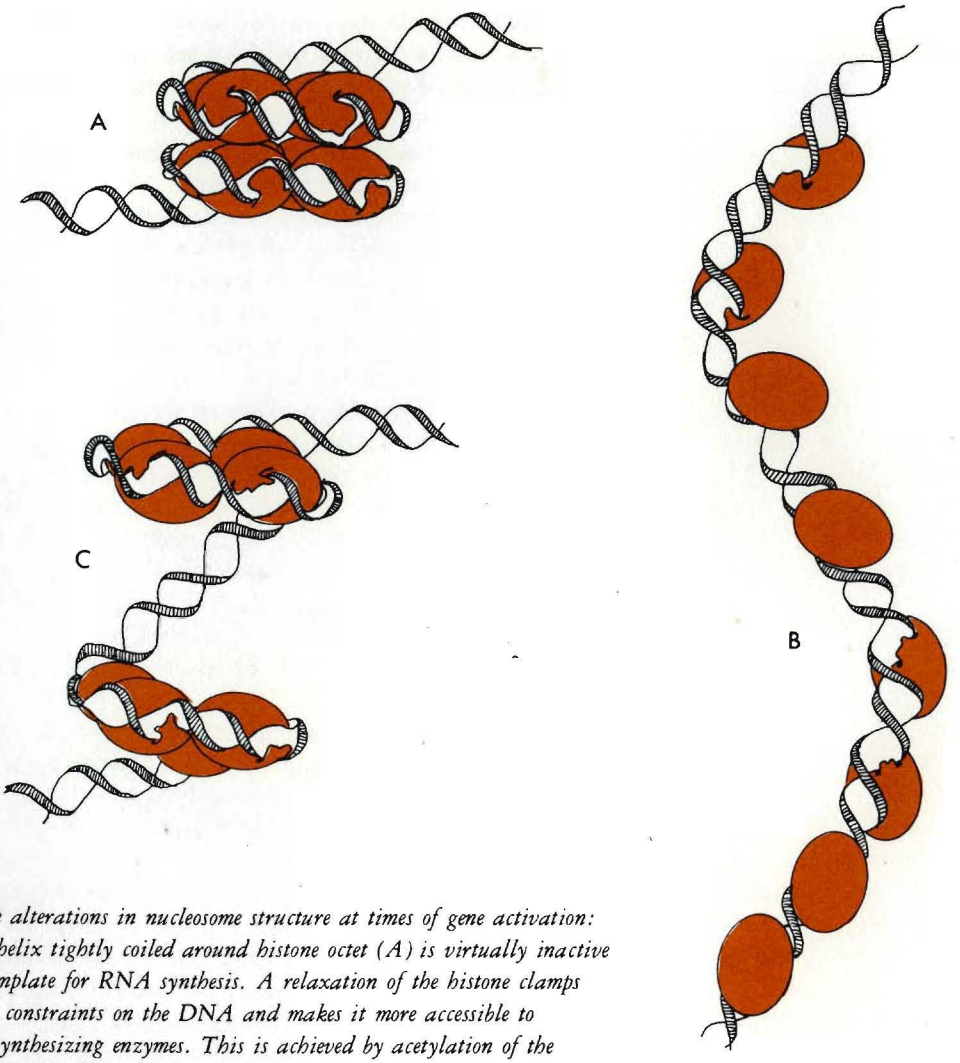
In the technique called gel electrophoresis, radioactively charged molecules (proteins, RNA, DNA) migrate through a viscous medium (gel) to different positions, forming distinct bands. The location of the bands identifies the molecules and the intensity of the radioactivity in each band measures the number of labeled molecules. Dr. Giorgio Vidali works with an electrophoretogram scanner designed by two Rockefeller physicists and a graduate student in chemical biology. Their new instrument greatly speeds the process—15 minutes as opposed to days or weeks—and improves its accuracy.

matin—the chromosome's mixture of DNA, histones, and other proteins—from mammalian cells, and he provided the first evidence that all the cells (except ova and spermatozoa) in the body of an individual of a species contain the same amount of DNA. Years later, the Mirsky laboratory, of which by then Vincent Allfrey was a member, announced the results of experiments confirming that RNA was the agent that copies and transports the message of DNA within the cells.

PROTEINS AND GENES: A KEY DISCOVERY

Dr. Allfrey was invited to join the Mirsky lab in 1949 after he had completed his Ph.D. at Columbia University. "From the start with Dr. Mirsky," he recalls, "my interest was in chromosomes and proteins. There were a number of laboratories, here and elsewhere, doing work on protein synthesis in the ribosomes, but ours was the first to get into nucleoprotein synthesis. I always smile when I think back to that time and about the details of research that don't get into the scientific papers. Some of the experiments we were doing required large organs from which we could extract a lot of nuclei. We got some of them from a slaughterhouse in New Jersey. I remember once I was coming back carrying a box with a horse liver in it, wrapped in waxed paper—we didn't have plastic in those days. It started to leak all over the Third Avenue elevated subway. People would come into the car, sit down next to me, notice the trail of blood, and move away pretty fast.

"From the work on protein synthesis, I got interested in histones. The first suggestion that histones might have a role in gene regulation came in the early 1950s. By that time, a number of laboratories were working on the problem. In time, people began to deduce that histones depress RNA synthesis. In 1964, my group made a discovery that really began to put the whole story together. What we discovered was a



Possible alterations in nucleosome structure at times of gene activation: DNA helix tightly coiled around histone octet (A) is virtually inactive as a template for RNA synthesis. A relaxation of the histone clamps releases constraints on the DNA and makes it more accessible to RNA-synthesizing enzymes. This is achieved by acetylation of the DNA-binding domains of each histone molecule. The result may be an uncoiling of the nucleosome (B) or possibly an unfolding into symmetrical half-nucleosomes. (C)

post-synthetic modification in the histones and we knew it had to have something to do with transcription." (By "post-synthetic" Dr. Allfrey means a change in the protein *after* it's been made and shipped into the nucleus.)

Histones and other nuclear proteins interact with DNA because the proteins' basic amino acids, like lysine, carry a positive charge which binds electrostatically with negatively charged phosphate groups on the DNA molecule. The protein and the DNA molecules lock together. Dr. Allfrey and his colleagues discovered enzymatic mechanisms that neutralize the positive charge on the histones and permit release of the constraints on the DNA helix.

"The reaction we discovered was acetylation," Dr. Allfrey says. "That is, the enzyme introduces an acetate group onto a lysine component of a histone and wipes out its positive charge. As the enzyme moves, zipper-fashion, along the DNA-binding region of the histone molecule, many lysine residues are modified and the histone may fall away from the DNA, leaving the DNA accessible, free to perform whatever function it needs to. An increase in acetylation of the histones is usually followed, within half an hour, by an increase in RNA synthesis.

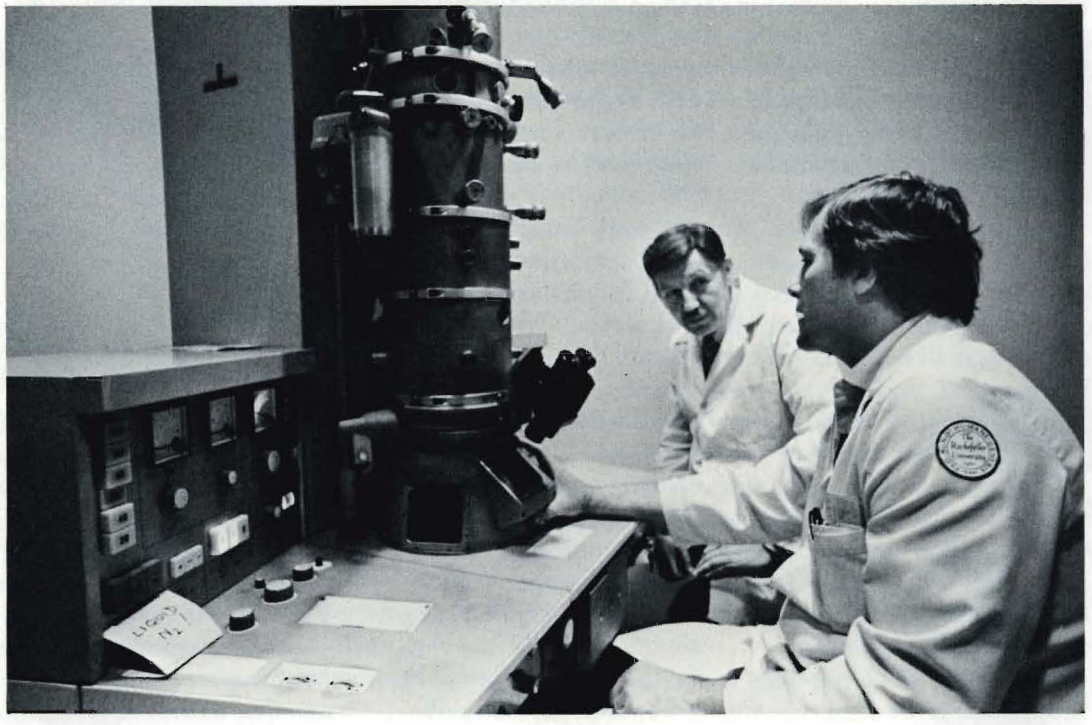
"In retrospect, it sounds simple but it took several years of work before we had it figured out. In 1968, my colleagues Giorgio Vidali and Edward Gershey—Ed was still in graduate school—and I discovered that the modification involved the formation of N-acetyl lysine. With that, the whole chemical picture became clearer. Until then we knew only that acetylation correlated with gene activity—it was an early event in gene activation—but we didn't know why. We could only guess. Finally we had proof that a modification of the lysine residue neutralizes the charge, chemical proof that you relax the constraints on the DNA by acetylating the associated histones. We've been following the ramifications of that ever since in one form or another."

Acetylation is only one kind of chemical activity affecting the nuclear proteins. There are others, more complex, called phosphorylation, discovered in the Allfrey lab in 1966, and methylation.

NUCLEAR PROTEINS AND CELL MALIGNANCY

"Given the evidence that these processes affect both the structure and function of the chromatin in normal cells," Dr. Allfrey continues, "it was natural to ask whether the proteins of the nucleus are involved in cell malignancy. We started by studying the nuclei of epithelial cells in the colons of rats injected with the chemical carcinogen, 1,2-dimethyl-hydrazine (DMH). An early and progressive increase in the synthesis of TNP-1, a specific nuclear protein class, was detected long before there were any morphological indications of malignancy, that is, changes in the cell structure. Similar proteins were found in studies of the nuclei of human colon cancer cells. So, now we are studying these proteins and their role in chromosome structure and function to seek to produce antibodies that might make possible early detection of colonic cancer. This is important for treatment to be effective.

"Another aspect of our cancer work was stimulated by a 1974 report on the use of urea to inhibit skin cancer and to extend the survival of patients with liver cancer. We speculated that sodium cyanate often present in urea might be the tumor-inhibiting component. Dr. Lidia Boffa and I tested cyanate with our colonic-tumor rats and found that it did selectively inhibit protein synthesis in the tumor cells without any appreciable effects on the animals' normal tissues. We discovered that the cyanate must first be metabolized in the animal's liver to produce an active anti-tumor agent. But we want to know how the cyanate works selectively against tumor cells. Here we get back to acetylation.



Dr. Allfrey (left) with Dr. Hans-Peter Hoffmann, at the electron microscope.

"Histone acetylation occurs very early in the course of gene activation in cells that are responding to hormones and other developmental stimuli. Conversely, the removal of acetate from histones by enzyme action correlates with gene inactivation in a number of cell types. Recently we showed that a simple organic acid salt, sodium butyrate, inhibits the action of enzymes that remove the acetate group from histones. This disrupts the normal dynamic balance between acetate uptake and release, and cells exposed to butyrate gradually accumulate hyper-acetylated histones. These cells often show altered gene activity such as abnormal hormone or enzyme production, and tumor cells exposed to butyrate lose many of their malignant growth characteristics.

"The butyrate-induced change in histone structure is essentially freeing up the DNA—making it more accessible. You can see the chromosome changes in the electron microscope. As a result, you suddenly get an activation of many of the genes that were previously being expressed at low level or not at all.

"We've used this phenomenon as a tool to analyze the cyanate effect. We've found that tumor cells placed in butyrate for a while are not sensitive to cyanate any more. However, the process is reversible. So, what we have now is an experimental system to observe these changes. We can actually look at changes that are significant in the expression of malignancy by varying the butyrate concentrations—putting it in and taking it out of the medium.

"In other words, what we're trying to do is to look at all these nucleoproteins with the new techniques in order to determine which proteins appeared, which disappeared, what changes occurred, how and when they responded to various stimuli, and then tie the whole thing together.

"We certainly didn't start out with any plan to put histone acetylation and cyanate sensitivity together and relate it to cancer, but that's how it seems to be working out. I guess that's how science is." □

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