experienced a recent heart attack. Our recommendation would be that people who had a recent heart attack be started on this drug prior to their discharge from the hospital. We could possibly extrapolate to people 3 months after their heart attack but we do not have the information to deal with the question of whether people should start taking propranolol if they had a heart attack as long as 5 years ago." Goldstein said it was up to doctors' clinical judgment to decide when heart attack patients should stop taking propranolol, if ever.

The results of the propranolol study, Friedewald concludes, "are an exciting breakthrough. Until now, we had nothing for patients who survived a heart attack. Now we have a drug that reduces mortality by 26 percent. That's an astonishing figure."—GINA BARI KOLATA

Genes Regulated Through Chromatin Structure

Unusual areas of chromatin upstream from genes are necessary for gene activity

In the electron microscope, all DNA from cells of higher organisms looks pretty much the same. Sections of the DNA containing active unique sequence genes cannot be told apart from inactive regions of DNA. Yet, clearly, there must be something that distinguishes transcriptionally active regions of DNA and that something, it is becoming apparent, is structure. Molecular biologists are uncovering evidence of structural differences in active regions of DNA and, in particular, are finding an unusual conformation upstream from active genes that appears to be necessary for transcription.

It comes as no surprise that DNA structure is so important for gene activity because DNA in cells of higher organisms is not merely a simple double helix. The DNA is virtually covered with proteins and when spread in electron micrographs, looks like beads on a string—the beads being balls of histone proteins with DNA wrapped around them and the string being DNA with still other proteins attached to it. This complex of DNA and proteins is called chromatin.

But, despite the likelihood that chromatin structure holds clues to gene activity, until very recently only a few molecular biologists focused on it. The problem was a lack of techniques to study gene expression in vivo. What most molecular biologists did was to strip the DNA of its proteins and, using methods that work so well for bacteria, transcribed the DNA in vitro, looking for sequences that may be necessary or sufficient for the regulation of gene expression.

In the opinion of many researchers in the field, the previous development of major significance in studies of chromatin structure occurred about 5 years ago, when investigators tried to solve the beads-on-a-string structure, and quickly succeeded. Since no one knew how to probe chromatin structure any further, they went on to other sorts of experiments. "Structure gets boring without function," explains Harold Weintraub of the Hutchinson Cancer Center in Seattle. Richard Axel of Columbia University agrees. "We're just getting back to chromatin structure. We waited until we knew where on the chromatin to look."

What helped turn many molecular biologists back to chromatin structure was, first, advances in recombinant DNA technology that allow them to pick out specific genes or chromatin regions containing genes, study or even alter the sequence, and then, if desired, reinsert the DNA sections into cells to examine their chromatin structure in vivo. The second impetus to look at chromatin structure was a finding by Weintraub that the enzyme DNAase I can more easily cut active than inactive regions of chromatin. Apparently, transcriptionally active regions of DNA have a more "open" or more "relaxed" structure that makes them more accessible to this enzyme. This was the first evidence that active genes really are structurally distinct.

Using this nuclease, Weintraub and his associates discovered that active globin genes and the chromatin surrounding them in chicken red blood cells are ten times more sensitive to the enzyme than the bulk of transcriptionally inactive chromatin. Inactive globin genes in red blood cell precursors or in brain cells, in contrast, are not particularly sensitive to the enzyme. The sensitive region in the red blood cells contains the globin genes and is about 100,000 bases long; the globin genes themselves are only 1000 to 2000 bases long.

Then, Carl Wu, now at Harvard University, followed by other researchers, began finding hot spots for DNAase I—short stretches, consisting of 100 to 200 nucleotides, that are 100 times more sensitive to the enzyme than are the average stretches of chromatin. These hot spots can show up nearly anywhere, it seems, appearing before genes, after genes, within genes, and even in areas of chromatin where there seem to be no genes at all. But what is most intriguing is that the hot spots that appear in front of genes seem to have a connection with gene activity.

As molecular biologists began looking for these so-called DNAase I hypersensitive regions, they found that every time a gene is active, a hypersensitive region can be found upstream from the gene.

So far, the hypersensitive regions have been found upstream from active chicken globin genes, active rat insulin genes, active yeast genes involved in mating, and a number of active *Drosophila* genes, including several heat shock genes, insulin genes, and a gene coding for glue protein. The hypersensitive regions, moreover, seem to be in a highly unusual conformation. Because hypersensitive regions have been found upstream from such a wide variety of active genes, Sarah Elgin of Washington University concludes, "It is safe to assume that if a gene is capable of being transcribed, it must have a hypersensitive region at its 5′ end."

Having gotten this far, molecular biologists are asking what happens to gene expression if a hypersensitive region is deleted or mutated, when during tissue differentiation do these hypersensitive regions appear, what do the regions look like, and how are the regions related to certain DNA sequences that seem important for gene expression in vitro.

The most dramatic evidence of what happens if a hypersensitive region is deleted comes from work by Mark Muskavitch, now at Harvard University, and Steven Beckendorf at the University of California at Berkeley. Muskavitch isolated *Drosophila* mutants whose larvae do not make glue protein, a protein

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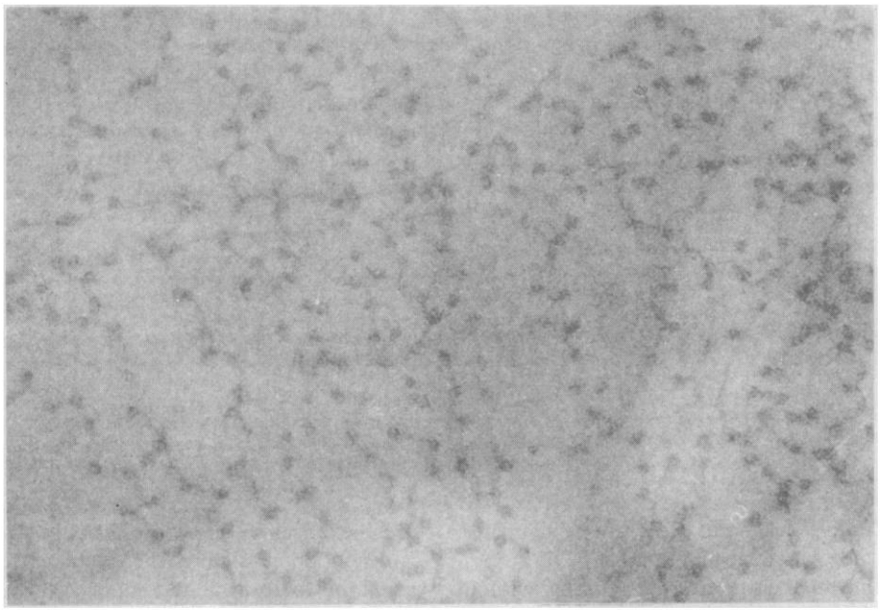

Donald Olins, Oak Ridge National Laboratory

Chromatin fibers streaming out of a chicken erythrocyte nucleus.

out by the larvae as they become pupae. The defect, Muskavitch found, was not in the glue protein gene itself but was a deletion 400 nucleotides upstream from the start of the gene. Beckendorf then showed that what was deleted was, in fact, a hypersensitive region. This result shows that the hypersensitive region is necessary for transcription rather than merely a consequence of it.

But the hypersensitive regions do not seem to be sufficient for transcription. For example, Weintraub finds that the regions appear in front of globin genes before the genes are capable of being transcribed and Elgin finds that the same is true for heat shock genes.

Weintraub uses cloned chicken red blood cells to determine the time during differentiation that the hypersensitive regions appear in front of globin genes. He takes red cell precursors and transforms them with avian erythroblastosis virus, which has a temperature-sensitive transforming gene. At low temperatures, the cells infected with the virus are arrested in their precursor states. At higher temperatures, they go on to differentiate and produce globin. Weintraub found that the hypersensitive regions appear two stages in their differentiation before the cells start to produce globin.

Heat shock genes are entirely different from globin genes. Unlike globin genes, they can be turned on in any sort of cell but they are expressed only during physical stress, such as high temperature. Although all animals, from *Drosophila* to humans, have these genes, their function is unknown. According to Elgin's work, the heat shock genes of *Drosophila* always have hypersensitive regions in front of them, even when they are not being expressed.

Because the hypersensitive regions are hot spots for enzymes that cut DNA, they must be in a configuration that is more open or more exposed than ordinary chromatin. Several investigators, using very different kinds of evidence, argue that the DNA in these regions is more relaxed and less likely to be covered with proteins than the rest of chromatin.

As evidence that the DNA in hypersensitive regions is in an unusual conformation, Weintraub cites two kinds of experimental results with the globin gene. First, he finds that the hypersensitive region of active globin genes can be cut by S1 nuclease, an enzyme that cuts single-stranded DNA and does not normally cut chromatin. He suspects that the DNA in this region may be unwound. Second, Weintraub finds that when he inserts the hypersensitive region from active globin genes into a supercoiled plasmid, the region acts as though it is unwound.

Kim Naismyth of Cold Spring Harbor Laboratories also finds that a hypersensitive region of chromatin from yeast DNA is, apparently, in a more relaxed configuration than the rest of the chromatin. In yeast, when a protein called mar binds to a hypersensitive region in front of a mating type gene, the gene is expressed. By putting the gene and its hypersensitive region in a supercoiled plasmid Nasmyth found that mar seems to unwind the DNA of the hypersensitive region.

As soon as the hypersensitive regions were discovered, an obvious question arose: How, if at all, are these regions related to two DNA sequences that are upstream from genes and are thought to be important for gene transcription?

The first of the two sequences, TATA, has been found in front of about 60 different genes and has been shown to be essential for gene transcription in vitro. The TATA sequence is between the gene and the hypersensitive region.

The second sequence, CCAAT, is within the hypersensitive region. It is thought to be important because it has been found in several different species. Since CCAAT apparently is conserved during evolution, it is reasoned, it must have some function. Because it is upstream from genes, it may function in gene regulation.

Axel asked what would happen in mouse cells if the TATA sequence preceding the thymidine kinase gene of herpesvirus were deleted but the hypersensitive region was left intact. The answer, he finds, is that the deletion has no effect—the thymidine kinase gene is accurately and efficiently transcribed in vivo. In fact, Axel finds that he can delete the entire 37 nucleotides between the thymidine kinase gene and the start of the hypersensitive region and still see no effect on gene transcription in vivo. He reports that as long as he leaves the hypersensitive region of the thymidine kinase gene intact, he can get efficient and accurate transcription of any sequence he places in front of it.

Steven McKnight of the Hutchinson Cancer Center has been constructing mutations within the hypersensitive region preceding the herpesvirus thymidine kinase gene and observing their effects on gene transcription in mouse cells. He is especially interested in the effects of mutations in the center of the region because that is where a CCAAT sequence occurs. "I expected the largest effect at CCAAT, but the largest effects [of the mutations] were at just the opposite positions, at either end of the region," McKnight says.

It is reasonable to speculate, says Weintraub, that the hypersensitive regions are sites on chromatin that are especially accessible to proteins used to initiate gene transcription. "We have established that chromatin structure is important. Genes are not just controlled by sequences at their 5′ ends. This sort of regulation through chromatin structure is different, interesting, and unique," Weintraub remarks. "Now we are at a crucial point. Our questions are getting clearer and soon there will be some sort of basic understanding emerging."—GINA BARI KOLATA