# New Clues to Gene Regulation

## *Enhancer sequences seem to be involved in turning on genes and may themselves be regulated by a small group of proteins*

It is a central question in molecular biology today, so fundamental that it sounds almost trite. What turns on specific genes at specific times in differentiated cells? The problem is at the heart of developmental biology, it lies behind research on the molecular basis of cancer, and it must be resolved if gene therapy is ever to come into widespread use. And although the question is far from being answered, it is beginning to yield. Investigators now have a glimpse at mechanisms that may underlie gene regulation and what they see so far hints that a variety of seemingly unrelated research may be tied together.

During the past few years, "enhancers" have been found. These sequences come before or after active genes and seem to turn them on or substantially increase their activity—thus the term "enhancers." And one group of investigators has isolated a protein that may be necessary for the enhancers to work.

The gene regulation story began about 3 years ago when Pierre Chambon and his associates at the University of Strasbourg School of Medicine and, independently, George Khoury and his associates at the National Cancer Institute discovered a repeated sequence, 72 base pairs in length, that seemed to be necessary for the expression of the "early" genes of the monkey tumor virus SV40. These early genes are the first to be expressed when the virus enters a cell. At the time he and Chambon made their discovery, says Khoury, "neither of us fully appreciated how significant the sequences are."

But soon afterward, Walter Schaffner and his colleagues at the University of Zurich found a similar sequence in the DNA of another tumor virus, polyoma virus, and Khoury and Barbara Levinson of the NCI found such sequences in retroviruses, the cancer-causing RNA viruses. Within a short time, a host of similar viral sequences were discovered by investigators at a number of different laboratories. As they studied how the sequences activate viral genes, the researchers began to find that the sequences are able to turn on genes whether they are located before or after the genes and whether they are in the normal orientation or are inverted. Moreover, some of these enhancers also seem to work when they are as far as 5 to 10 kilobases from the genes they are turning on, they can turn on virtually any gene they are near, and the major factor that seems to determine whether they turn on a gene is what sort of DNA sequences lie between the enhancer and the gene. Other genes that are put in between, for example, can damp out an enhancer's effects.

Still, these were effects of viral gene sequences. No one had any reason to suspect that enhancers had anything to do with normal, tissue-specific, cellular gene expression. It seemed reasonable, however, to at least look for enhancer-like sequences in cellular DNA. Although different viral enhancers have grossly different nucleotide sequences, they seem to share a core of nucleotides. A number of groups of researchers looked for and found such "enhancer consensus sequences" in cells of humans and laboratory animals. But what was needed was a demonstration that these or other sequences actually turn on specific genes.

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The first good evidence that enhancers might normally activate cellular genes came last summer when three groups of investigators independently found that enhancers turn on immunoglobulin genes in antibody-producing cells (*Science*, 19 August 1983, p. 735). Six months later, Michael Walker, Thomas Edlund, Anne Boulet, and William Rutter at the University of California in San Francisco discovered enhancer-like sequences that turn on insulin genes in insulin-producing cells and other enhancer-like sequences that turn on chymotrypsin genes in cells that produce this enzyme.

The insulin and chymotrypsin enhancers, says Rutter, are similar to the other enhancers discovered so far in that they work, to some extent, whether they are in front of or behind the genes and they also work if their orientation is reversed. But their DNA sequences are unexpected. "Their sequences bear no resemblance that we can tell to the sequences of the other enhancers," he remarks. This means that, for the time being at least, researchers looking for enhancers will have to do more than simply screen for an enhancer consensus sequence. Such sequences, even if they are found, may not turn genes on.

The next question is, How do these enhancers work? The assay for the insulin and chymotrypsin enhancers is based on the assumption that there is a soluble molecule, a protein perhaps, that recognizes the enhancer and interacts with it in some way. For example, a protein found only in insulin-producing cells might bind to the insulin enhancer and change the structure of the chromosome in the vicinity of the insulin gene. As a result, RNA polymerase might be able to bind and start copying the gene.

Until very recently, this hypothesis was just that—a hypothesis. No one had ever found an enhancer-recognizing protein for a cellular gene and no one had any good way to go about searching for one. But now Gary Felsenfeld and his colleagues at the National Institute of Arthritis, Diabetes, and Digestive and Kidney Diseases have found a protein with many of the characteristics of an enhancer-recognizer. "This is the first glimmer of hope," says Khoury. "In my opinion, they may well have come upon an enhancer recognition protein."

Felsenfeld has long been intrigued by changes in chromatin structure that occur when genes are active. Normally, DNA is coated with proteins and wound around small histone proteins so tightly that it is virtually inaccessible to nucleases that would otherwise degrade it. But when genes are turned on, the structure of the chromatin containing them changes so that it now is accessible to nucleases.

About 3 years ago, Felsenfeld and his associate James McGhee found a nuclease-hypersensitive region in chicken β-globin genes. This region, they discovered, is hypersensitive only when the erythrocytes are from chickens that are at least 9 days old, which is when the β-globin genes begin to be expressed. Erythrocytes from younger chickens have β-globin genes that are resistant to nucleases.

Felsenfeld and McGhee began to focus on this region, isolating it from erythrocytes with restriction enzymes. The nuclease-hypersensitive region, they discovered, is 115 base pairs long and is located upstream from the globin gene. Then Felsenfeld and his colleague Beverly Emerson decided to look for a protein that leads to nuclease hypersensitivity. If there is such a protein, they expected it might bind specifically to the 115-base-pair region that is exposed whenever the globin gene is active.

They found such a protein, which appears only in erythrocytes and only when globin genes are active and makes the globin gene hypersensitive to nucleases. "We hope that this will turn out to be a transcriptional factor," says Felsenfeld. "So far it fits." But he and his colleagues still have more experiments to do, particularly studies to determine whether the nuclease-hypersensitive region to which the protein binds is an enhancer sequence. "We're very excited and we hope—that's all," Felsenfeld remarks.

Of course, in a sense, all that the enhancer work has done is to push the question of tissue-specific gene activity back a step. Instead of asking why globin genes, say, are turned on in erythrocytes at a particular time, researchers are asking what determines where and when a gene coding for a globin gene enhancer recognition protein is turned on. If every gene that is turned on has to have its own enhancer recognition protein, the problem of understanding gene regulation will be at least as difficult as it appeared before enhancers were discovered. "We hope there's some sort of degeneracy," Felsenfeld says.

Most of the investigators are betting that there are only a small number of these regulatory proteins. During differentiation, they believe, there may be some means of turning on a small set of genes coding for these proteins and this would lead to the activation of genes such as globin or insulin that distinguish one cell type from another. "The important thing is that it really does focus our attention on an aspect of differentiation that was unknown to us before, says Rutter. "We don't have the secret to differentiation but we have a powerful way to study it. This is by no means the end of the story but it is certainly a beginning."—**GINA KOLATA**

# Blooms in the Desert?

As researchers continue to sift through data from the proton-antiproton collider at the European Laboratory for Particle Physics (CERN)—data that last year resulted in the discovery of the long-sought W and Z particles—they have begun to encounter hints of something utterly new and unexpected. The particle physics community is correspondingly excited: the anomalous events occur at energies just beyond the W and Z mass, where the so-called grand unified theories of particle interactions had predicted a "desert" in which nothing new will be found.

"The desert," CERN's Carlo Rubbia told the American Physical Society (APS) during its meeting in Washington, D.C., last month, "is blooming."

Anomalies were reported at the meeting from both of the collider's main detectors, UA1 and UA2, albeit the anomalies are different in each case. None of the events seem to be explainable in terms of the standard unified theory of the electromagnetic and weak interactions, which predicted the W and Z particles. In particular, none of them seems to be either a top quark or a Higgs boson, which are the only remaining undiscovered particles in the theory.

The UA1 puzzle involves five events in which a proton-antiproton collision produces a highly collimated "jet" of charged particles shooting out to one side, while an unseen object—presumably a neutral particle of some kind—recoils in the opposite direction. Jets, of course, are ubiquitous in high-energy particle collisions; the problem is that these jets have an energy of 50 to 80 GeV, which is much too high to have been produced by a W particle. One and possibly two additional events feature overenergetic photons instead of jets.

The unseen neutral objects may well be neutrinos, concedes Rubbia, although they would have to be produced by some novel mechanism. But more exotic objects are also possible, such as the photino, a heavy partner to the photon predicted by theories based on supersymmetry (*Science*, 29 April 1983, p. 491).

Rubbia also noted another puzzle, perhaps related: too many Z particles appear to be decaying into multiple jets. Theory suggests that each additional jet should be suppressed by a factor of about 100, which should make four- and five-jet events rare indeed. But they are not rare.

CERN's Jean-Marc Gaillard told the APS meeting of a slightly different effect found at the UA2 experiment. In three or possibly four events that would otherwise look like the normal production and decay of a W, the decay products—an electron and an unseen neutrino—are accompanied by extraordinarily energetic jets. Gaillard declined to speculate on new particles or unknown phenomena. However, the events are *very* far from anything else seen in connection with the W's and Z's and essentially impossible to explain in the standard model.

In addition, the UA2 researchers have found preliminary suggestions of enhanced production of jets with an energy around 147 GeV. This might indicate the existence of a new particle of the corresponding mass.

The evidence in both experiments is admittedly arcane and fragmentary. Everyone would like more statistics. But for particle physicists used to dealing with such esoterica the evidence is intriguing. "It is suggestive of something important happening at 150 to 200 GeV," says Alfred K. Mann of the University of Pennsylvania, and a nonparticipant in the CERN experiments. "There is a sense of optimism that we are entering a region of mass—accessible to existing machines—with interesting new physics."

The April announcements have certainly served to whet people's appetites for the next experimental run at CERN, which starts in September and continues for 3 months thereafter. The major target will be the top quark, for which the detectors have been optimized. But both the energy and the beam luminosity will be higher than in previous runs, which should greatly improve the statistics for rare events. Moreover, the detectors will be computerized to the point that the researchers will know about events within hours, instead of months. The findings, if they come, should be dramatic.—**M. MITCHELL WALDROP**