Biology

Julie Ann Miller reports from Philadelphia at the Third Annual Congress for Recombinant DNA

Enhancing research on gene regulation

The rules by which genes turn on and off remain a major mystery to biologists, and biology still seems to have cards up its sleeve. A recent glimpse into the puzzle revealed a new genetic element—one that dramatically increases a nearby gene's activity. So far various examples of this element, called an enhancer, have been discovered among the genes of different animal viruses, and scientists expect to find such control elements among cellular genes. Enhancers have some intriguing, and so far inexplicable, properties. Peter Gruss of the University of Heidelberg in West Germany described investigations of enhancers in his and several other laboratories in Europe and the United States. Enhancers also are being used to encourage expression of genes transferred by scientists into mammalian cells.

The enhancer region has been found to be a short stretch of DNA. In some cases copies are repeated one after the other. The enhancer's properties contrast with those of the promoter, a DNA region recognized earlier to control activity of a gene. The promoter, which is considered the gene's start signal, must lie adjacent to the gene at the end where expression begins. The enhancer typically lies about 100 base pairs away from this end, but it is still active in experiments where it has been separated from the gene by as many as 4,000 base pairs, turned backwards or even placed at the other end of the gene.

An enhancer region will work to increase activity of any gene it is placed near, even such cellular genes as globin. However, an enhancer seems to function best in the cells normally infected by the virus from which it comes. In at least one case described, a viral gene is under several layers of control. Its activity can be induced by a hormone, and its enhancer works only when the hormone is present. But if the piece of DNA responsible for the hormone action is removed, the enhancer can then exert its effect without hormone. "The induction sequence somehow interferes with enhancement function," Gruss told SCIENCE NEWS.

Scientists are now comparing different enhancers, both natural and altered in the laboratory, to determine what gives them their power. They find a core of about eight nucleotides common to functional enhancers from a wide variety of viruses, Gruss says. He suggests the most likely explanation of enhancer function is that it aids recognition of a gene's start signal by the enzyme responsible for initiating gene expression. Alfred Nordheim of the Massachusetts Institute of Technology reports experiments suggesting that short stretches of an unusual DNA form known as Z-DNA (SN: 1/9/82, p. 24; 12/22 & 29/79, p. 420) are present in an enhancer and may play a role in its function.

Heavy metal: Genes behind cell resistance

Throughout the plant and animal kingdoms, cells make small proteins that bind heavy metals, such as copper and cadmium, and thus protect themselves against toxic effects. In all species so far examined, the genes for the proteins (called metallothioneins) are turned on by the same metal ions that the proteins bind. These genes are providing scientists both with control regions useful as tools for regulating other genes and with an opportunity to investigate the basis of gene control.

The most dramatic genetic engineering use of one of these control regions has been the transfer of a rat growth hormone gene into mice (SN: 12/18 & 25/82, p. 389). The pattern of expression of the transferred gene with a mouse metallothionein control region follows that expected for metallothionein, says Ralph L. Brinster of the University of Pennsylvania. In other work, Michael Karin of the University of Southern California School of Medicine has used the control region of a yeast gene for copper resistance. A stretch of as few as 140 base pairs can be fused to other genes to make them turn on in the presence of copper.

The activity of some metallothionein genes is regulated both by heavy metals and by glucocorticoid hormones. Karin has examined one such human gene (out of about a dozen human metallothionein genes). A length of 770 base pairs adjacent to the gene contains the sites of both types of control, he finds. Karin reports that fusing this segment with a foreign gene makes the hybrid gene responsive to both heavy metals and hormones. This regulatory sequence can exert its action over long distances, like an enhancer does, Karin says.

In contrast, Dean Hamer of the National Institutes of Health, who is analyzing metallothionein genes of mice, monkeys and humans, has isolated genes with a shorter stretch of regulatory sequence, such that they respond to heavy metals but not to hormones. He finds a length of DNA about 30 nucleotides long that affects regulation by metal but does not alter the gene's basal level of activity. Hamer says his result indicates metals and hormones regulate this gene by independent means.

The details of this gene control are now under intensive investigation. Both Brinster and Hamer suggest that the secondary structure—a stem and loop arrangement of 30 to 50 nucleotides in the control region—is important to the activation of the gene by metals. Hamer finds this regulatory region a good place to investigate gene control. He says, "It's not exotic but it's amenable to detailed analysis." Karin agrees, "The main limit now is our imagination."

New tricks with synthetic sequences

Detecting human genetic diseases, identifying a member of a gene family and creating specific mutations in DNA are all possible using chemically synthesized strings of nucleotides about 20 bases in length, reports R. Bruce Wallace of the City of Hope Research Institute in Duarte, Calif. These feats are possible because under certain conditions the stability of a pair of DNA strands requires a perfect match (adenine to thymine and guanine to cytosine) at every position along the chain. For example, to detect the gene for sickle cell anemia in a patient, Wallace and colleagues synthesize short strands of nucleotides mimicking the section of the gene that has a single nucleotide difference between the normal and sickle cell genes. The normal gene binds preferentially to the synthetic strand with the normal sequence; the sickle cell gene binds to the nucleotide strand with the characteristic sickle cell sequence. Scientists are applying this technique to other human genetic diseases including β^0-thalassemia and α1-antitrypsin deficiency. The method should be applicable wherever the genetic abnormality, however small, is known. Previous techniques required the abnormality to fall at a site cut specifically by an enzyme (SN: 7/10/82, p. 23).

Chemically synthesized nucleotide chains also are useful in identifying a specific gene among a family with many similar members. The sequence of the chain can be determined from the amino acid sequence of the protein whose gene is sought. Because in the genetic code there are cases in which more than one set of three nucleotides encode a single amino acid, Wallace synthesizes a mixture of all possible coding sequences. Using this method, he made a 19-nucleotide chain that binds to the single gene, out of a family with more than 20 similar members, for a membrane component (called H2-K^b) involved in immune system recognition. "I think this will be an important strategy for looking at multi-gene families," Wallace says.

Finally, Wallace uses his synthetic molecules to direct specific changes in large pieces of DNA. He can change single base pairs or delete any region. "Now one can pretty much do anything," Wallace says. For example, he has made a 21-nucleotide chain that pairs with part of a gene for a transfer RNA molecule. The pairing causes a specified region of DNA, in this case the intervening sequence, to loop out and consequently be deleted from the gene. Wallace says such experiments can investigate the function of intervening sequences.