Immunoglobulin Genes Have Enhancers

Such enhancers may help regulate cellular gene expression in development; they may also contribute to cancer by activating genes abnormally

The mysteries of gene control in the cells of higher organisms are gradually yielding to the techniques of molecular biology. A recent case in point is the discovery in immunoglobulin genes of enhancer elements, DNA sequences that markedly increase the transcription of genes into messenger RNA, the first step of protein synthesis.

The discovery is the first unequivocal demonstration that enhancers, which were originally identified in the genomes of viruses, are found with cellular genes. It helps to define the signals needed for gene activation and may lead to a better understanding of the regulation of gene expression during development.

Many genes, including those of the immunoglobulins, are activated only in particular tissues or at certain times. The basis of this differential expression is largely unknown, but the new work suggests that some enhancers are signals that can only be recognized in the right type of cells. "The implications of enhancers may be even larger than we expected," says George Khoury of the National Cancer Institute (NCI). "There is a hint from the immunoglobulin story that you can have tissue-specific activation, the differential turning on of genes." The immunoglobulin enhancer works in cells that normally make immunoglobulins but not in cells that do not.

Finally, the immunoglobulin gene enhancer and perhaps other cellular enhancers may contribute to the development of cancer by inappropriately turning on the cellular counterparts of viral oncogenes (onc genes). These genes, which are thought to be involved in normal growth and differentiation, may come under the influence of active enhancers as a result of the chromosomal rearrangements occurring in many types of cancers.

The identification of the immunoglobulin enhancer solves a puzzle about the activation of immunoglobulin genes during the development of antibody-producing cells. A few years ago, molecular biologists learned that the genes that code for the protein chains of which complete antibody molecules are composed must be assembled from shorter segments of DNA. The gene for the lighter of the two chains is formed by first bringing together two DNA segments, which are designated V (for variable) and J (for joining) to produce the coding region (exon) for the variable region of the light chain. This remains separated from the constant region exon by a large noncoding intron. The intron is eventually removed by RNA splicing from the messenger RNA (mRNA) for the light chain, thus joining the variable and constant region sequences that specify the complete light chain.

The assembly of the heavy chain genes is similar except that the variable region exon is formed by joining three DNA segments. The third, which is designated D (for diversity), is located between the V and J segments.

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Antibody-producing cells do not make complete immunoglobulin chains until after the V-J or V-D-J rearrangements have taken place. This raised a question about the nature of the signal that activates expression of the genes. The DNA sequences that were thought to be involved in initiating transcription of DNA into mRNA are located upstream from the beginning (5′) end of the V region segment. But this 5′ flanking region is not altered by the DNA rearrangements, except that it is brought much closer to the constant region exons and the intron immediately preceding them.

Meanwhile, investigators were looking for enhancer sequences associated with cellular genes. They had been found in a number of viruses, including simian virus 40 (SV40) and polyoma virus, both of which are DNA viruses, and in the long terminal repeats found at the ends of the genomes of the retroviruses, which have RNA genomes but which replicate through a DNA intermediate.

Studies from a number of laboratories, primarily those of Paul Berg at Stanford University School of Medicine, Pierre Chambon of the University of Strasbourg School of Medicine, Walter Schaffner at the Institut für Molekularbiologie II der Universität Zürich, and of Khoury at NCI, established that viral enhancer elements have some unusual properties. They can activate transcription when placed either upstream or downstream from the 5′ end of the gene. And they can do it even when their orientation is inverted and the reverse of what it usually is. The enhancers work with genes from sources other than their own. Finally, they can increase transcription when located some distance away from the beginning of the gene, perhaps as much as several kilobases.

Since upstream control sequences did not seem to be involved in activating rearranged immunoglobulin genes, a downstream enhancer element would be a good candidate for such a role. Two groups report in the July issue of *Cell* unequivocal evidence for the presence of an enhancer in the intron between the V-D-J coding region and the C region exons of the rearranged heavy chain gene.

Stephen Gillies and Susumu Tonegawa of the Massachusetts Institute of Technology (MIT), with Sherie Morrison of the Columbia College of Physicians and Surgeons and Vernon Oi of Stanford University School of Medicine transferred a cloned heavy chain gene into a line of mouse myeloma cells that had lost the ability to make a heavy chain. After introduction of the complete cloned gene, the cells made large quantities of the heavy chain. If Tonegawa and his colleagues deleted the intron between the V-D-J and C coding regions before transfer, very little of the protein was produced. The decreased synthesis was the reflection of a reduction in transcription of the transferred gene into mRNA.

Walter Schaffner and his Zürich colleagues, Julian Banerji and Laura Olson, used a somewhat different approach to show that the large intron of the heavy chain gene contains a segment that enhances transcription. They cut the cloned intron into three fragments by digesting it with a restriction enzyme and then linked each of the fragments separately either to the gene coding for the T antigen of SV40 or the β-globin gene. They found that one of the fragments

stimulated the production of mRNA transcripts of the genes.

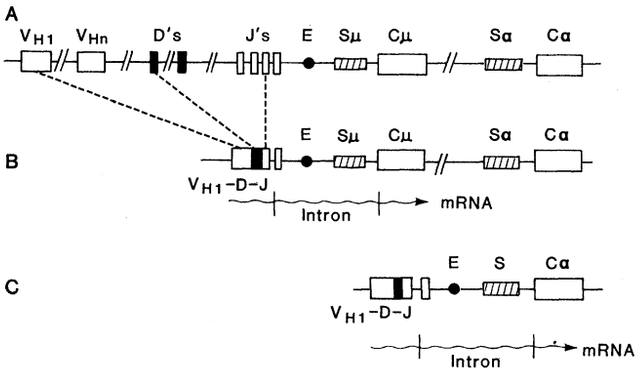
A third group, Mark Mercola, Xiao-Fan Wang, Jory Olson, and Kathryn Calame of the University of California at Los Angeles, also finds strong evidence for an enhancer in the large intron of the heavy chain gene, as they report in the 12 August issue of *Science*. They attached cloned intron segments to a bacterial gene and showed that the segments increased production of the gene product in a line of monkey kidney cells.

Just showing that a DNA segment increases transcription does not prove that it is an enhancer, however. The sequence must have the other properties of these elements. All three groups went on to show that the intron segment does display those characteristics. As Tonegawa puts it, "The experiments satisfied all the requirements for an enhancer. It is essential for transcription, it can be moved around or inverted, and it works with heterologous genes as well."

Light chain genes may also carry enhancer sequences in their large introns. A paper in the July *Cell*, by Cary Queen, who is now at NCI, and David Baltimore of MIT, deals with this possibility. These investigators showed that a cloned light chain gene is transcribed into mRNA when transferred into mouse myeloma cells. However, when the constant region exon, plus about one-third of the intron immediately preceding it, was deleted from the gene, no transcription could be detected, indicating that the deleted DNA contains a regulatory sequence needed for transcription. But Baltimore says, "We can't say that it is an enhancer yet." They have not shown that the regulatory sequence has the additional properties required of a true enhancer. Nevertheless, he notes, it is reasonable to expect enhancers in light chain genes, as well as in those of heavy chains.

The location of the enhancer element in the large intron of the heavy chain gene is the logical one, as is that of the putative enhancer in the light chain intron. To obtain correct initiation of transcription the enhancer must interact with the previously mentioned control sequences that are located upstream from the beginning of the V region segment. Before V-D-J or V-J joining, the upstream and enhancer sequences are far apart; but the rearrangement brings them close enough together for transcription to occur.

The heavy chain enhancer is also located in the right place to activate transcription for the heavy chains of all five major classes of immunoglobulins.



Enhancer element in heavy chain gene. The germ-line arrangement of the heavy chain gene is shown in diagram A. The enhancer element (E) is between the segments coding for the J portion of the variable region and the switch (S) region for the first constant region exon (Cμ). Diagram B shows the arrangement after joining of the V, D and J segments. This brings the control sequences to the left of the V region exon much closer to the enhancer, thus allowing transcription to produce mRNA for the heavy chain. The large intron is spliced out of the original transcript before the messenger is translated into protein structure. The switch from one immunoglobulin class to another involves the deletion of the C region exons preceding the one to be used, a rearrangement which is mediated by the switch region. As shown in diagram C, the enhancer remains in the appropriate location to facilitate transcription after the class switch.

These are formed by attaching a rearranged V-D-J sequence to any of five different C region segments.

During development of an antibody-producing cell, the V-D-J segment is first attached to the Cμ exon to form the heavy chain for an immunoglobulin M. Later the cell may switch to producing one of the other classes of immunoglobulins. The class switch requires the deletion of all the C region exons before the one to be used. This deletion is mediated by a repeated segment of DNA located just before each C region exon. Because the enhancer element is located between the 3′ end of the V-D-J segment and the switch region for Cμ, the first C region exon, the enhancer will be left in place even after the class switch and thus can continue to activate transcription.

The immunoglobulin gene enhancer is tissue-specific for cells that can produce antibody. In fact, early attempts to locate enhancers in immunoglobulin genes, which were done before reliable methods for transferring genes into antibody-producing cells were available, had failed because investigators had used the wrong cell types.

While the enhancer for the heavy chain gene works very well in mouse myeloma cells, Tonegawa and his colleagues found that it has little stimulatory effect on transcription of the gene in mouse fibroblasts. The Zürich workers obtained similar results and also showed that the enhancer increases expression of the T antigen gene in a line of antibody-producing cells of human origin. "That means that the enhancer fragment can be active in immunoglobulin-producing cells of different species, but is not active in the same species if we use fibroblasts," Schaffner explains. He and his colleagues had previously failed to obtain expression in HeLa cells.

The observation by the UCLA workers of enhancement in monkey kidney cells does not necessarily militate against the proposed specificity of the heavy chain gene enhancer. They used a line of transformed cells that allows the vector in which the bacterial gene and enhancer sequences are transferred to replicate, thus producing many copies per cell.

Enhancement of transcription may involve the activity of cellular factors that specifically recognize only certain enhancer sequences. But if a large number of copies of the enhancer are present, even a recognition factor that binds to it very weakly may be able to stimulate detectable transcription. No enhancement was detectable, the UCLA workers note, in the untransformed parent cell line where the vector does not replicate.

There had been earlier indications that certain viral enhancers were at least species-specific. For example, the Schaffner group and also Khoury, Laimonis Laimins, and their colleagues at NCI, had shown that viral enhancers worked preferentially in cells of one species but not in those of another. More recently, Queen and Baltimore found that control of the light chain gene is very different in a line of monkey kidney cells from that in the mouse myeloma cells. In the kidney cells its transcription was much reduced and not affected by deletion of the downstream sequences.

All in all, the results suggest that some cells, but not others, contain factors that can recognize specific enhancers. In keeping with this hypothesis is the observation that the nucleotide sequences of enhancer elements from various sources differ from one another, although they often contain a core sequence GTGGXXXG, where G represents the base guanine and X can be either adenine or thymine.

The exact size of the heavy chain gene enhancer has not been delineated. The Tonegawa group finds that most of the enhancing activity is contained in a fragment of some 140 base pairs. The Zürich workers suggest that sequences needed for enhancement are distributed throughout the 300 base pairs of their active fragment. The fragments do contain the core sequences, however.

The manner in which enhancers stimulate transcription is unknown. The sequences may serve as sites of entry for the enzyme that transcribes DNA into RNA. This could be done in a relatively nonspecific way if the elements simply altered the chromatin structure in their vicinity to make it more open to the enzyme. The binding of the enzyme could be made more specific if the proposed recognition factors help to direct it in some fashion to the right gene, possibly by serving as a component of the enzyme itself.

In addition to turning on gene expression in an orderly way during development, enhancers may also do so inappropriately, perhaps leading to the development of cancer. Certain of the retroviruses can apparently activate cellular onc genes by inserting their long terminal repeats, which carry enhancer sequences, near the genes.

In tumor cells from many patients with Burkitt's lymphoma, the chromosome rearrangement characteristic of the cancer moves the cellular myc gene near the switch region for the Cμ coding segment where it may come under the influence of the heavy chain enhancer. The chromosomal translocation, Khoury points out, "may have contributed a red hot transcriptional region to a gene that is either quiescent or well-regulated otherwise." However, in other tumors, such as mouse plasmacytomas, the myc gene is translocated to a new position near the Cα segment, where there is no known enhancer. Although the presence of an enhancer there has not been ruled out, the UCLA workers did not find any enhancing activity in the DNA between the Cα exon and its switch site.

The issue of whether the specific translocations associated with various cancers lead to the development of malignancy by causing the overproduction of a normal gene product or because the translocated gene undergoes some change resulting in an altered product has not been resolved. Perhaps both contribute.

The supposition is that other genes, in addition to that for the immunoglobulin heavy chain, have their own enhancers. There are indications that enhancer-like sequences are found in the cellular genome. Khoury and Nadia Rosenthal of NCI and Michael Botchan and his colleagues at the University of California at Berkeley have identified sequences which are structurally related to viral enhancers and which increase gene transcription. Looking for additional specific enhancers will be high on the agenda of molecular biologists. Another item to be given high priority is the development of the cell-free systems needed for establishing how enhancers work.

The research has the potential of not only providing a better understanding both of normal development and of malignancy, but may also lead to improved control of genes that are introduced into new cells in gene transfer experiments. Poorly regulated expression of such genes, those for the globin chains, for example, has been a problem. Use of appropriate enhancer sequences may improve control of the genes and perhaps facilitate development of methods for treating hereditary diseases by gene therapy.—JEAN L. MARX

Predators and Hurricanes Change Ecology

Results from direct experimentation in natural communities has reemphasized the importance of predators and climate in community organization

Ecologists are in the midst of a major—and frequently acrimonious—debate about the processes in nature that influence the structure of communities. For more than two decades the phenomenon of competition between species has been prominent—and some say completely dominant—in ecologists' thinking about the way ecological communities are shaped. Indeed, as Jonathan Roughgarden, of Stanford University, puts it, "Competition theory was the only theory on the block."

During the past several years this establishment position has come under sharp attack from several directions. On one flank a group of workers, led by Daniel Simberloff of Florida State University, has challenged the validity and interpretation of data that many ecologists claim support competition theory (see last week's issue, page 636). Attackers on the other flank proselytize experimental manipulation, as opposed to field observation, as the superior method of obtaining data that might more reliably reveal the biological processes underlying patterns in community organization. This approach, which has been inspired largely by Joseph Connell of the University of California, Santa Barbara, has highlighted the effects of predation and environmental change in determining community structure, at the expense of competition between species.

The reaction against the hegemony of interspecific competition has been accompanied by something of an antitheory shift in ecology. Donald Strong, a colleague of Simberloff's at Tallahassee, sees benefits in this movement. "The dethroning of competition has provoked a vigorous empiricism of the most productive sort." Roughgarden, however, notes in a forthcoming paper in *American Naturalist* that "There is a curious tone of righteous indignation in Strong *et al.* (1979) and of ridicule in Connell (1980) that is antitheoretical." Strong retorts, "What we want is a realistic theory, not no theory."

One reason why interspecific competition so rapidly and decisively reached its prominent position in ecological thinking was the mathematical elegance and apparent explanatory power of the late Robert MacArthur's theoretical models, which he developed during the 1960's. Like many ecologists of the time, and since, who were interested in community ecology, MacArthur studied the distribution and morphology of bird populations. Irregularities in the distribution of bird species—species A never coexists with species B, for instance—has become something of an exemplar in competition theory, but it is an exemplar over which there are now keen differences of opinion (see last week's article).

A second reason for the swift ascendancy of competition theory is rooted in the philosophically appealing notion of