# Biochemical Studies on Gene Expression in Higher Organisms

*by Carl Parker*

A chemical biologist is trying to gain an understanding at the molecular level of the mechanisms controlling gene expression

ONE OF THE MAJOR GOALS in modern biology and biochemistry is to understand exactly how gene expression is controlled in higher organisms. It is well known that genes are the basic unit of the material of inheritance. We know that each individual organism receives a set of hereditary information from its parent or parents, replicates it during growth or preparation for reproduction, and passes it on — usually largely unchanged — to its progeny. At present, there is very little understanding of how a single cell develops into a complex multicellular organism. It is very likely that the expression of genes at different times in different cells brings about, to a large extent, the development of a complex multicellular organism. Thus, the central question is, How is it that these genes are selected only at certain times in a select subset of cells?

My research interests are to gain an understanding at the molecular level of the mechanisms controlling gene expression. We know that there are several points at which gene expression can be controlled. The first level of gene expression, called transcription, involves the precise copying of the information contained in DNA (deoxyribonucleic acid) into another long polymer called RNA (ribonucleic acid). This process is mediated by a rather complex, multi-component enzyme called RNA polymerase.

The next level of gene expression is called translation. During translation, the RNA molecule produced by transcription is read by complex, multi-component entities called ribosomes to polymerize amino acids into proteins. Proteins in turn can be modified by other proteins in the cell, and thus a third level of the regulation of gene

expression is evident. In bacteria, where the molecular processes governing gene expression are becoming well defined, we know that the control of gene expression is effected at the level of transcription to a large extent. Thus, my research group is focusing its attention on transcriptional control mechanisms in higher organisms.

My research group currently consists of my research assistant, Joanne Topol; three graduate students, Warren Kibbe, Bette Korber, and Gil Scott; an undergraduate student, Doug Ruden; a postdoctoral fellow, David Price; and one technician, Terry Koch. We are taking a biochemical approach toward understanding transcriptional control mechanisms in eukaryotes. Our attention is focused on two organisms in particular: *Drosophila melanogaster* (a fruit fly) and *Saccharomyces cerevesiae* (bakers' yeast).

Drosophila is a particularly useful organism for biochemical studies of transcriptional control, since it possesses a group of eight genes that respond to the simple elevation of the temperature at which the fly is growing. These so-called heat-shock genes respond to elevated temperature by a dramatic increase in their levels of transcription and translation — to over a hundredfold more than they were at room temperature. The heat-shock genes, therefore, serve as an excellent model system for our studies aimed at discovering how genes turn on at the level of transcription.

Several other research groups have actually isolated all of the Drosophila heat-shock genes by using recombinant DNA technology. They have gone one step further to completely determine the DNA sequence (the sequence of nucleotides making up the DNA molecule and gene) of many of the heat-shock genes.

At Stanford, where I was a postdoctoral fellow with David Hogness, I developed a procedure for preparing RNA polymerase from Drosophila such that the enzyme would recognize the appropriate DNA sequence near a gene (a region called the promoter) and transcribe the gene correctly *in vitro*, that is, in the test tube. It was already known that a purified RNA polymerase from Drosophila was not capable of specifically starting transcription at the correct site *in vitro*. Thus, it was necessary to use a crude preparation of RNA polymerase in order for a biologically meaningful reaction to occur in the test tube.

My research group at Caltech is seeking to understand just what other proteins or "factors" other than RNA polymerase are required for initiation of transcription *in vitro*. It is our hope that by identifying these factors and learning how they function we will be better able to study the molecular processes that regulate gene transcription. We have, by a combination of approaches, identified three different components (in addition to RNA polymerase) required for the initiation and termination of transcription *in vitro* of several Drosophila genes. Increasing evidence is emerging that one of these factors binds to the promoter, a sequence of the DNA template that is known to be critical for transcription. The other two components are not as well characterized, but the preliminary evidence leads us to speculate that one of these two components interacts with the RNA polymerase. In fact, we have observed, by various biochemical procedures, that a complex of all three factors and RNA polymerase can be discerned. Should the transcription complex that we see in the test tube exist in the cell, one may conjecture that this complex is capable of initiating transcription on a wide variety of genes, provided the gene has either the appropriate structure or necessary accessory proteins to make that gene active.

In addition to the perhaps more general transcription factors I have just described, Joanne Topol and I have identified another factor that possesses a very interesting property. This protein(s) binds specifically to a DNA sequence that is known from experiments in other laboratories to be required for the transcriptional regulation of one of the heat-shock genes of Drosophila. This is a particularly exciting result because it allows us, for the first time, to begin experiments aimed at exploring the mechanism of transcriptional activation of a gene from higher organisms.

The next step beyond these experiments will be to actually isolate the genes for the more general transcription factors and the specialized heat-shock regulatory site binding protein. This can be achieved by several approaches including the advanced technology developed in Leroy Hood's laboratory involving the synthesis of a particular region of the gene based on its determined amino acid sequence. It is easy to imagine that we can at that point ask detailed questions about the regulation of gene expression of genes whose products control expression of other genes — thereby stepping up in the hierarchy of control of gene expression.

The facilities in the Braun Labs are not going to make our work any less complicated or demanding, but it should be physically easier to accomplish our research goals because of the excellent resources available there. We are fortunate also to be supported financially by the National Institutes of Health, the Camille and Henry Dreyfus Foundation, the Sloan Foundation, and the generosity of the Division of Chemistry and Chemical Engineering at Caltech. □