# Control Element Found Within Structural Gene

## *A puzzle about certain gene regulation in the bacterium* Escherichia coli *is resolved by the discovery of a novel location of a control element*

A group of molecular biologists at the National Institutes of Health has discovered an entirely novel genetic arrangement by which the expression of certain genes in the bacterium *Escherichia coli* is controlled. Typically, on a strand of DNA control elements precede the structural genes that they regulate. In the newly discovered pattern, however, the control element, which in this case is known as an "operator," is located within the coding sequence of a structural gene.

In bacteria, all the genes are pretty much accessible to the enzymes that copy them into messenger RNA (mRNA). The problem, then, is not so much how to turn genes on as it is how to turn them off when they are not needed.

There is a standard model to explain control of gene expression in bacteria. It is the "operon" and consists of a set of genes that are regulated together. Usually, an operon starts with a "promotor" region, which is the binding site for the polymerase that copies the structural genes into mRNA. After the promotor comes an "operator" region, which frequently overlaps with the promotor and is used in shutting off the genes of the operon. Finally, there are the structural genes themselves.

When the genes of an operon are turned on, RNA polymerase binds to the promotor and begins copying. To turn off the genes, a protein, called "repressor," binds to the operator and physically prevents the polymerase from binding.

This model explains how a number of clusters of bacterial genes are regulated. But there is a notable exception. The *gal* operon, which contains a group of genes coding for proteins that metabolize the sugar galactose, is different. Instead of lying between the promotor and the structural genes, the operator lies upstream from the promotor and there is no overlap. "The operator," says Sankar Adhya of the National Cancer Institute, "is too far out. It was not clear how simple binding of repressor to the operator could prevent binding of RNA polymerase to the promotor."

The only idea that researchers had was that repressor binding somehow changes the DNA configuration upstream—an action-at-a-distance model. But it was not at all clear what sort of configurational changes would occur or why they would prevent RNA polymerase from binding to the promotor.

Adhya and his colleagues Meher Irani, who is now at the University of Washington, and Laszlo Orosz, who is now at Attila Jozsef University in Szeged, Hungary, were intrigued. They isolated and sequenced mutations in the *gal* operator and looked for clues to how the system works. But some of the mutations were a mystery. They clearly were operator mutations but they did not seem to lie in the operator region of the *gal* operon.

Finally, the three molecular biologists decided to sequence the entire *gal* operon, looking for the sites of these operator mutations. To their great surprise, they discovered a second operator region within the first structural gene of the *gal* operon. The baffling mutants had alterations in this second operator.

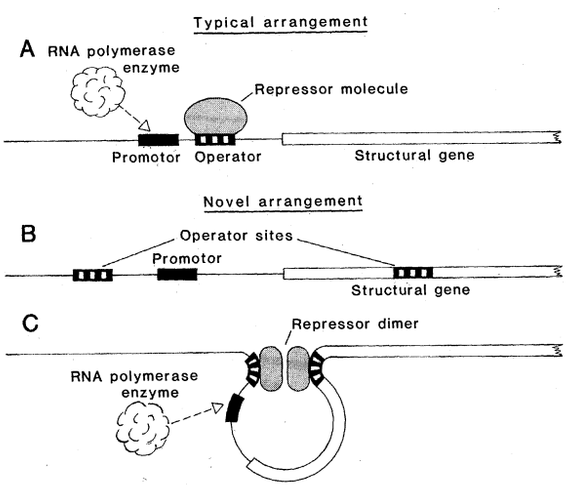
The second operator is oriented in the opposite direction from the first. What seems to happen, Adhya says, is that a molecule of repressor binds to each of the operators. The two repressor molecules then bind to each other to form a dimer and the promotor region in between loops out. (The active *gal* repressor is known to be a dimer.) Thus the RNA polymerase is prevented from binding to the promotor.

The result, says Adhya, "is interesting because people think protein binding changes DNA, not necessarily in the immediate vicinity but over some distance. This may be the first example of such DNA changes with demonstrated biological value in vivo."

The next question the NIH scientists asked themselves was, Is this sort of system peculiar to the *gal* operon or is it more general? After going back and thoroughly searching the literature, they found reports that the *lac* operon of *E. coli* might have two additional operator sites. Nearly 10 years ago, researchers doing repressor binding studies found a second site just before the first structural gene of the operon and a third site immediately upstream from the *lac* promotor. But the *lac* repressor binds to these sites with 10 to 100 times lower affinity than it binds to the primary operator site and the two "pseudo-operators" have not been properly tested for their potential role in the functioning of the *lac* operon in vivo.

Adhya does think, however, that the sort of molecular mechanisms for inhibition of gene expression found in the *gal* operon with its two operators might be more generally applicable. One possibility is that a similar system might be employed by yeast. "The difference between stored and active sex-determining genes in yeast turns out to be that the stored genes have special sequences flanking them. And protein binding at those two sites causes structural changes of the DNA in between which makes the genes active," Adhya says.

But, for now, this system of inhibiting gene activity in bacteria is only known to apply in the *gal* operon. Nonetheless, this intriguing result demonstrates once again that even in bacteria, surprises can occur.—**Gina Kolata**




***Operon alternatives***

*Binding of a repressor molecule to an operator site in the normal operon arrangement (A) blocks binding and progression of the RNA polymerase. In the* gal *operon (B) two repressor molecules bind to two operator sites, which then form a dimer. The promotor site is looped out and transcription is blocked (C).*

**Additional Reading**

1. M. H. Irani, L. Orosz, S. Adhya, *Cell* **32**, 783 (1983).