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## Genes Can Answer to More than One Master

Like discovering a car that has more than one engine under the hood, cell biologists are learning to their surprise that alternate molecular machines can drive the basic process of transcription that orchestrates the expression of genes.

The core transcription machinery of RNA polymerase copies the information found in DNA genes onto messenger RNA molecules that then govern the production of proteins.

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— Robert Tjian

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Although the reason for multiple transcriptional controls remains mysterious, researchers speculate that the mechanism might allow the same gene to be used for different purposes in different cells.

Now, Howard Hughes Medical Institute (HHMI) researchers have taken an important step in understanding this phenomenon by pinpointing the first gene in the fruit fly *Drosophila melanogaster* that is a target of an alternate control molecule, called TRF1. They believe that the discovery opens the way for a richer understanding of how gene expression is regulated.

In an article in the May 5, 2000, issue of the journal *Science*, HHMI investigator Robert Tjian and graduate student Michael C. Holmes report that the *Drosophila* gene *tudor* contains tandem promoter segments, one of which responds to TRF1.

"The discovery of TRF has been intriguing because for perhaps the last fifteen years we thought that the basal transcriptional machinery of the cell

was essentially invariant," said Tjian, who is at the University of California, Berkeley. "We thought that only one set of "general" proteins was involved, and that all the regulation was directed by enhancer-binding proteins that were specific to a particular gene sequence.

"It was like using the same engine over and over again, but just putting different gearing systems into it. But then we discovered that there were multiple engines."

The scientists had found evidence that TRF1 is apparently one of several alternate transcriptional control molecules called recognition factors that can replace the most prevalent control element, called TATA-binding protein, or TBP.

"While past studies had proven that TRF1 was involved in transcription, the big question was why was it particularly exciting just finding another TBP-like molecule," said Tjian. "But then research revealed, surprisingly, that this molecule was not evenly distributed in every cell. Some cell types, particularly those in the central nervous system, expressed high levels of this protein and others had either very low levels or none at all."

To attempt to pinpoint a particular TRF1-responsive gene from among the 12,000 known *Drosophila* genes, the researchers first launched an "aerial reconnaissance" of *Drosophila* chromosomes. Using a technique called polytene chromosome staining, they created an antibody that specifically targeted and attached to TRF1. They then bathed the giant salivary chromosomes from *Drosophila* in the antibody. Since the antibody also included a staining molecule, they could home in on potential TRF1-targeted genes by scanning the fly genome for regions that were preferentially stained.

"We found that only about forty or fifty bands on the fly chromosomes lit up," said Tjian. "This told us that our hypothesis that TRF1 was specialized for certain genes was on the right track."

To find the TRF1-responsive genes, the scientists treated preparations of fly chromosomes with chemicals that formed bonds between TRF and the DNA. They then chopped up the chromosomes into small pieces and identified those pieces that had attached to the TRF1-specific antibodies.

Using the pieces of chromosomes as clues, they were able to work their way up to identifying whole genes. Screening those genes revealed that the *Drosophila* gene *tudor* is a potential target gene that can be activated by TRF1. To validate the responsiveness of *tudor* to TRF1, the scientists cloned the promoter region of *tudor* and tested whether it responded to TRF1 *in vitro*.

"The result of this test was more interesting than I anticipated," said Tjian. "We thought that these genes would either have a TBP-responding promoter or a TRF-responding promoter. But *tudor* had both tandem promoters. I think this is perhaps the most unexpected piece of data in the paper."

According to Tjian, the discovery of tandem promoters represents the opening of a new terrain for the exploration of transcription control.

"Right now, trying to explain these tandem promoters is total speculation," he emphasized. "However, if you look at the genome of the fly, it's about 12,000 genes. In contrast, the roundworm, *C. elegans*, has about 18,000 genes. Now, the fly is at least as complex, if not more complex than the worm, and one way to achieve that higher complexity with fewer genes is to make the same gene-coding capacity more versatile. One way this versatility could evolve is by simply having more elaborate control mechanisms over a smaller number of genes." Thus, said Tjian, the same gene might be governed by alternate control schemes in different cells.

Tjian and his colleagues plan to look for other genes that have multiple controls and to explore further this newfound diversity of gene control.

"The take-home lesson from these studies is that we're now appreciating more than ever before that the basic workhorse transcriptional apparatus is much more elaborate and probably more specific to organisms and tissues than we imagined," he said.