

A Game with Lots of Time-Outs

A new technique reveals that RNA polymerase takes several breaks as it does its essential gene-transcribing work.

IT ALMOST SOUNDS LIKE THE SETUP TO A PUNCH

LINE: How is reading a gene like playing baseball? But the answer is no joke. Just as baseball players stop at first, second, or third base on their sprint toward home plate, the molecules called RNA polymerases frequently pause at certain places along the DNA in the process of transcribing a gene.

Now, using a new technique, HHMI predoctoral scholar Kristina Herbert, working in Steven Block's lab at Stanford University, and her colleagues have pinpointed where on the double helix individual molecules hesitate in the act of transcription. In fact, certain places in the DNA underfoot seem to signal those mystery stops, they reported in the June 16, 2006, issue of *Cell*.

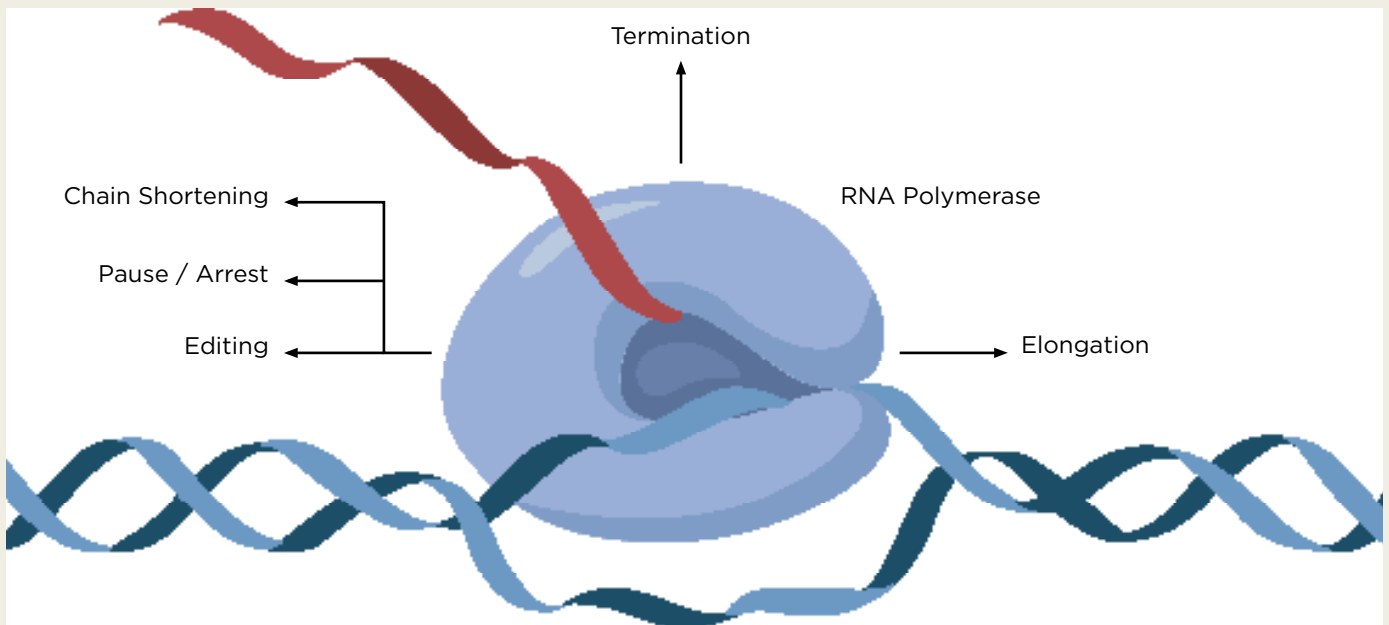
The excitement about the study has as much to do with the technological advance as it does with the finding.

"This is a major accomplishment," says Peter von Hippel, a chemistry professor at the University of Oregon, who wrote an accompanying commentary on the paper. "The job of the transcription complex is to read the sequence. You have to know exactly where you are on the [DNA] template in order to interpret the biological consequences of being there."

Genetic information flows from DNA to RNA to proteins, according to molecular biology's central dogma. The gene transcription enzyme, RNA polymerase, transcribes the code from DNA to RNA. With a DNA template strand in its grasp, the baseball-glove-shaped molecule reads the genetic instructions and creates a new, complementary strand of messenger RNA, which contains the code for building proteins.

"Gene expression is at the mercy of RNA polymerase," says coauthor Robert Landick, a bacteriology professor at the University of Wisconsin–Madison. "Its movement along the DNA as it's making RNA is heavily regulated by all kinds of factors in cells that control how fast or slow it moves and its propensity to keep going or fall off. That's a big deal for human genes—whether or not the RNA polymerase can make it through millions of base pairs to make a functional gene product."

Researchers in Block's lab specialize in single-molecule studies. They use a ray of invisible infrared light to grip and manipulate tiny beads linked to the molecules. A second laser detects the changing positions of the suspended beads as, say, RNA polymerase trundles down a strand of DNA. Several years ago, Block's team first observed



the stop-and-start progress of a single RNA polymerase along the DNA. Later, they fine-tuned the technique to show the polymerase's steps—less than one nanometer apart—from a single base pair to the next. They did not know where the molecule was exactly but saw that it was pausing—a lot.

Herbert wanted to know precisely where the polymerase was at all times, so she could learn more about the pausing. The easiest way to do that, she figured, was to program a well-known pause site into the double-stranded bacterial DNA. She created two DNA templates, each with eight copies of a pause site important for gene regulation, separated by the same repeating 240-base-pair sequence. She expected to see the polymerases dawdle at the preset sites, which would tell her where it was on the DNA. Instead, the polymerases made many more stops, and the pauses appeared indistinguishable. “I didn’t know which pause was the one I cloned in,” she says.

She and postdoctoral fellow Arthur La Porta, a coauthor, took advantage of the repetitive underlying DNA sequence to develop a computer algorithm to identify the genetic street address of each pause site and the behavior of the polymerases there. They analyzed 114 polymerase travels down the DNA templates.

They found that, like a baseball player rounding bases, a polymerase may halt, for different lengths of time, at specific DNA signals. And, like an anxious base runner, the enzyme may ignore signals to “hold up.” At any given pause sequence, however, some 30 to 80 percent of polymerases actually do pause.

Herbert is repeating her experiments with a laser beam that pushes, rather than pulls, on the polymerase bead to test how the different force affects pausing behavior and possibly to learn more about the underlying mechanisms. She is also experimenting with different transcription cofactors, looking for changes in pausing behavior at different sites. ■

— CAROL CRUZAN MORTON

FIGURE ABOVE MOST VALUABLE PLAYER: RNA POLYMERASE FULFILLS MORE THAN ONE ROLE

In addition to elongating messenger RNA, RNA polymerase takes part in a number of key tasks, such as editing and chain shortening. Pausing is the likely first step on any of these alternative pathways.