First Peek at DNA Transcription

For the first time, researchers have recorded molecular-scale images of the initial step in a process crucial to living cells—the use of genetic information contained in DNA.

Neil H. Thomson of the University of California, Santa Barbara and his coworkers have produced sequences of micrographs that show an enzyme molecule straddling a strand of DNA and pulling it along to complete the beginning phase of protein manufacture.

Thomson described the research last week at an American Physical Society meeting in Kansas City, Mo.

"We're seeing the opening of a new field of biophysics, based on locating, isolating, attaching, and manipulating single molecules," says Carlos Bustamante of the Institute of Molecular Biology at the University of Oregon in Eugene.

The researchers used a specially adapted atomic force microscope to capture the interaction between a strand of DNA and an enzyme known as RNA polymerase, obtained in this case from the bacterium *Escherichia coli*.

The polymerase initially binds to a DNA strand anywhere along its length, then slides like a bead on a string to find the sequence where it can start the transcription process. Snagging chemical building blocks known as nucleoside triphosphates (NTPs) from the surrounding liquid, the polymerase molecule reads DNA sequences to construct shorter strands called messenger RNA. In a subsequent step, the cell uses the information encoded in messenger RNA to produce a protein.

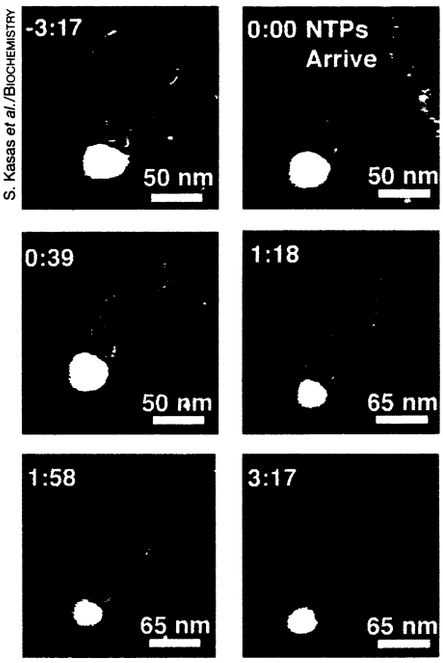
To image RNA polymerase bound to DNA, Thomson and his colleagues measured minuscule deflections of the sharp tip of a stylus as it moved back and forth across molecules pinned to a mica surface.

The entire assembly was bathed in a solution containing, at the start of the experiment, all but one of the ingredients essential for transcription. The polymerase began transcription but halted shortly thereafter, when it needed the missing building block. This pause allowed the researchers to focus on a single DNA-enzyme assemblage.

"We're actually imaging while we're flowing liquid through the system," Thomson says. "In this way, we can initiate a change by adding the appropriate chemical."

When the researchers introduced the final ingredient, transcription resumed and they observed the rest of the process. —*I. Peterson*

S. Kasas *et al.*/Biochemistry



In this sequence of atomic force microscope images, an RNA polymerase molecule (seen as a white blob) initially pins the middle of a DNA strand (top left). When NTPs, the ingredients necessary for transcription, arrive (top right), the polymerase starts copying the DNA, pulling it through to the right (middle and bottom, left). Finally, the polymerase releases the DNA strand (bottom right). The messenger RNA produced by the process is too small and mobile to be imaged. The elapsed time is shown in minutes and seconds (top left of each frame); length is measured in nanometers.

Eye pigments may depend on one amino acid

If a person walks into the bright outdoors from a dark movie theater, he or she may blink a little but can still see. That ability underscores how well the eye's two types of light-sensitive cells complement each other. The cylinder-shaped rods work best in dim light and reset slowly, while the cones, which make color vision possible, operate in brightness and reset more rapidly. Part of this specialization comes from the different pigments embedded in their membranes.

Now, it seems that a single amino acid in those pigments bears much of the responsibility for this difference in function.

Rod and cone pigments consist of light-sensitive molecules, or chromophores, held by proteins. Researchers at Kyoto University in Japan have found that by swapping amino acids between rod and cone proteins, they can confer rodlike properties on a cone pigment and vice versa. Their study appears in the March 18 Proceedings of the National Academy of Sciences.

The group focused on the rod protein rhodopsin and two cone proteins. All three proteins have somewhat different amino acid sequences but similar three-dimensional structures. The amino acids at three positions in rhodopsin are electrically different from the corresponding amino acids in the cone proteins.

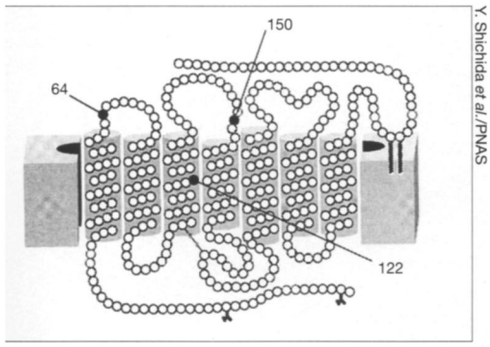
At position number 122 in the amino acid sequence of the proteins, for example, rhodopsin has a negatively charged glutamate, whereas one of the cone proteins has a neutral glutamine. Switching glutamine for glutamate in rhodopsin reduced the time the new pigment needed to return to normal after stimulation by light, bringing it much closer to the cone pigment's response.

"It's not quite a 100-fold effect, but it goes a long way toward that," says Clint L. Makino of Harvard University Medical School in Boston. Similarly, the reverse substitution in the cone protein increased the pigment's recovery time.

Swapping amino acids in the other two positions didn't change the response rate significantly.

The new study, Makino says, suggests that the amino acid at position 122 sits close to the chromophore. —*C. Wu*

Y. Shichida *et al.*/PNAS



The approximate location of the amino acids in rhodopsin as it loops through the cell membrane. Black circles indicate positions at which rhodopsin's amino acids have a different electric charge from those of cone proteins. Of the three positions, only 122 appears to affect the pigment's response to light.