Crystal Clear

X-ray snapshots illuminate how enzymes stitch together DNA

By JOHN TRAVIS

In 1953, James Watson and Francis Crick earned immortality in the annals of science by identifying the three-dimensional shape of deoxyribonucleic acid, better known as DNA—the chemical that makes up genes. In 1968, Watson set down his account of the race to this discovery in *The Double Helix*, a book whose title succinctly summarizes the structure of DNA.

This helical shape results from two intertwined strands of nucleotides, the building blocks of DNA. Watson and Crick argued that DNA's four nucleotides pair only in certain combinations—an adenine on one strand normally joins only to a thymine on the other strand, and cytosine pairs with guanine.

This complementary nature of the two DNA strands suggested a solution to a major mystery of genetics: How do cells copy their DNA?

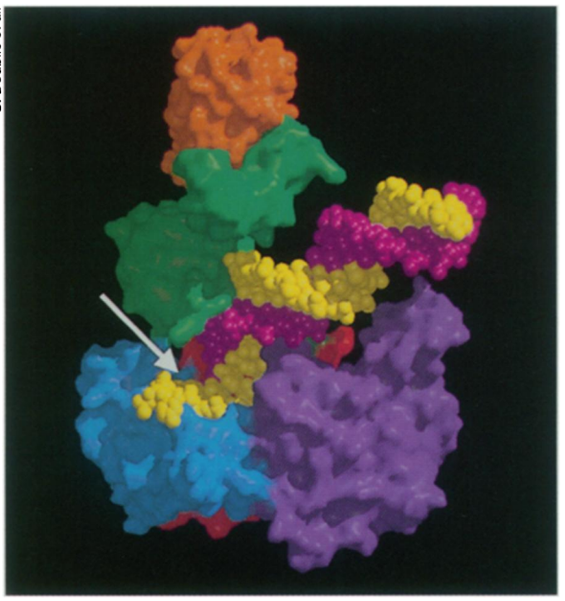
"It has not escaped our notice that the specific pairing we have postulated immediately suggests a possible copying mechanism for the genetic material," Watson and Crick noted in the remarkably reserved final sentence of their April 25, 1953 report in NATURE.

A month later, the scientists made public their proposal that DNA's double helix unwinds its two strands, and the cell then reads the nucleotides on each and fashions the new, complementary strands.

In the years since, researchers have largely confirmed Watson and Crick's hypothesis and have identified the tools that cells employ in this process. Central among them are DNA polymerases, the enzymes that choose appropriate nucleotides and stitch them together into new strands of DNA. Some polymerases repair short spans of damaged DNA, while others duplicate whole genomes at a time.

"These are the enzymes that make copies of the blueprint of life," says Lorena S. Beese of Duke University Medical Center in Durham, N.C.

Now, by shining X rays at crystallized versions of the polymerases, a research group led by Beese and one led by Tom Ellenberger of Harvard Medical School in Boston have obtained high-resolution images of the enzymes' three-dimensional structures. With these pictures in hand, the investigators have begun to clear up the mysteries surrounding how these crucial proteins work.



S. Doublié *et al.*

This computer model of the T7 DNA polymerase was created with data obtained from shining X rays through a crystallized version of the viral enzyme. The polymerase (red, green, blue, and purple) is bound to the DNA (pink and yellow strands intertwined in a double helix) and is poised to add a nucleotide at the end of the shorter of the two strands (arrow). A bacterial molecule called thioredoxin (orange) binds to the polymerase and helps it secure the DNA.

"The results provide atomic-level detail of an enzyme that is incredibly important for maintaining the stability of genetic information," says Thomas A. Kunkel of the National Institute of Environmental Health Sciences in Research Triangle Park, N.C.

"It's as if we're seeing frames from a movie, and if we get enough different shots, we will eventually get the whole story," adds Catherine M. Joyce of Yale University.

The story should be compelling, because polymerases often lie at the heart of gene mutations that can cause cancer or other diseases.

"One of the easiest ways for a mutation to arise is from a copying error by a DNA polymerase," says Kunkel. "It's really an important human health issue to understand how DNA is copied accurately."

Investigators have for many years created crystals of DNA polymerases and shone X rays through them to reveal the precise locations of the molecules' many atoms. While each polymerase has displayed its own unique shape, the images have inspired researchers to describe DNA polymerases in general as shaped somewhat like an open hand—that is, a palm, a thumb, and a set of fingers.

"Essentially, you have a flat part where the DNA sits and two appendages that kind of wrap around the DNA," explains Ellenberger.

Scientists believe that the palm holds a polymerase's active site, the region where the enzyme catalyzes the chemical reaction that joins a new nucleotide to a DNA strand. The DNA and the nucleotide bind to different regions of the polymerase, but the enzyme somehow brings them together in the active site.

Ellenberger and his colleagues may now have captured this climactic scene on film. In the Jan. 15 NATURE, they publish an X-ray picture with all three characters—the polymerase, the target DNA, and the incoming nucleotide—of this genetic drama. Their snapshot records a dramatic change in the shape of the polymerase that may help explain how it recognizes what nucleotide to add and then attaches it.

Some of the excitement surrounding the findings of Ellenberger and his colleagues stems from their investigation of a DNA polymerase that copies whole genomes at

a time. The polymerase is used by a bacteriophage, a virus that infects bacteria, to duplicate its genome during reproduction. This viral polymerase, called T7, even has a region that helps it proofread the DNA it has just created.

"It's a very simplified version of the replication systems that are found in mammalian cells and bacteria, yet it faithfully mimics many of the properties of these more complicated systems," says Ellenberger.

By itself, T7 can link only a dozen or so nucleotides before the DNA strand it is reading falls away from the enzyme. Yet when the virus infects bacteria, T7 steals a bacterial protein called thioredoxin that helps it hold onto the DNA. With thioredoxin's aid, T7 can polymerize thousands of nucleotides at a time, says Ellenberger.

To produce their X-ray picture of T7, the investigators created a crystal of the polymerase bound to a DNA fragment whose strands were of different lengths. When not in crystal form, the polymerase would normally extend the shorter of the two strands by adding nucleotides that complement the ones on the longer strand.

With the hope of freezing the polymerase in the middle of this process, the scientists allowed the crystal to form in a solution containing nucleoside triphosphates, the free-floating nucleotide precursors. They chose the guanine precursor because it would pair with the first available nucleotide, a cytosine, on the longer strand of the target DNA.

Most previous X-ray crystallography of polymerases bound to DNA showed the enzyme's hand in a relatively open configuration that made it difficult to understand how the polymerase could work. The T7 picture, however, has the polymerase's fingers in a dramatically different position. Like a hand making a fist, the fingers are rotated inward more than 40°, which seems to create a snug region where only the correct nucleotide can fit and then join to the shorter strand of the targeted DNA.

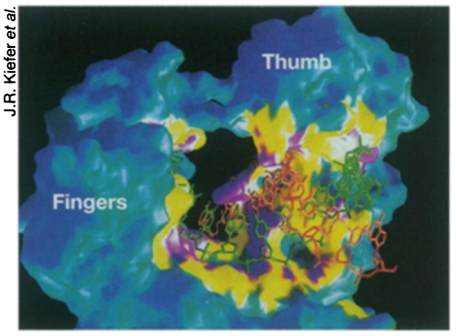
"We think the grip tightens and loosens" with every attempted addition of a nucleotide, says Ellenberger. "The fingers close around the active site of the enzyme, and that allows important residues to catalyze the incorporation of a nucleotide. Then the polymerase has to relax its grip so that the DNA can slide to the next position and the next incoming nucleotide can bind."

This grip-and-release mechanism must occur in a flash and with remarkable accuracy. T7 hooks together 300 nucleotides per second, making a mistake perhaps once in a million times.

Their analysis of how T7 works, add Ellenberger and his colleagues, strongly supports a proposal put forth several years ago by Thomas A. Steitz, a Howard Hughes Medical Institute investigator at Yale University. He suggested that two metal ions help bind an incoming nucleotide to the polymerase and facilitate its union with DNA.

Noting that she and other scientists have struggled to understand how DNA polymerases move a nucleotide and DNA into the proper positions for joining, Joyce calls the picture of T7 a "revelation." She says, "To me, the Ellenberger paper makes everything fall into place."

That's not to say that no mysteries remain. The researchers are still puzzling over how thioredoxin, which attaches to the thumb region of T7, helps the polymerase clamp so securely onto a DNA strand.



J.R. Kiefer *et al.*

This computer model of the bacterial polymerase studied by Lorena Beese and her colleagues shows the enzyme's fingers and thumb regions. In its largely hidden palm region, the polymerase has added nucleotides to a bound DNA molecule. Green and orange represent the DNA's two nucleotide strands.

If Ellenberger and his colleagues have captured a DNA polymerase at a critical junction of its work, Beese's group has high hopes of filming every detail of its job. In the Jan. 15 NATURE, Beese and her colleagues publish the highest-resolution picture yet of a DNA polymerase. Even more important, they report that their polymerase maintains its ability to link nucleotides despite being locked in the rigid structure of a crystal.

"That's an extremely important advance because it will enable us to look at a frame-by-frame snapshot of how nucleotides are incorporated," says Bruce W. Stillman of Cold Spring Harbor (N.Y.) Laboratory.

The polymerase under investigation by Beese's group is a newcomer to the field. Jeffrey C. Braman of Stratagene, a biotech firm in La Jolla, Calif., recently isolated it from *Bacillus stearothermophilus*, a bacterium that lives in hot springs in Idaho.

Following a strategy similar to that of Ellenberger's group, the investigators tried to take an image that showed the polymerase, a target piece of DNA, and a new nucleotide about to be added. Instead, the image showed only the polymerase bound to the target DNA. "Initially, we thought our experiment had failed . . . we were very disappointed," recalls Beese.

A closer look revealed that the shorter strand of the target DNA had gained the extra nucleotide, implying that the polymerase had done its job before the scientists took the picture.

This was unexpected, since few crystallized enzymes—and none the size of a polymerase—maintain their ability to catalyze chemical reactions. The researchers now have images of the crystallized polymerase after it has added four new nucleotides to DNA.

While these pictures don't show a distinct nucleotide as well as DNA, they nonetheless provide important insights. Capturing before-and-after pictures of the polymerase adding a nucleotide to bound DNA has cleared up a question about the direction in which the genetic material moves across the polymerase as new nucleotides are incorporated.

"There had been a lot of confusion about how DNA enters the polymerase and how the DNA is oriented relative to the catalytic site," notes Stillman.

In addition, the crystallized polymerase generally maintains its accuracy in pairing adenine with thymine and guanine with cytosine. "The enzyme doesn't seem to put in the incorrect nucleotide. Under normal conditions, it usually puts in the right one," says Beese.

The investigators have found certain conditions under which they can force their polymerase to mismatch nucleotides, and they hope to image those events to reveal why it is normally so selective.

Moreover, much as photographers turn to strobe lights to capture high-speed events, the scientists plan to use short bursts of high-intensity X rays to make a movie that will catch every step of the polymerase in action. Such a movie would probably contain a frame similar to that taken by Ellenberger's team, but other frames may reveal additional shape changes that are crucial to the polymerase's function, notes Beese.

Beese and her colleagues also plan to photograph their polymerase as it tries to copy DNA attached to known chemical carcinogens. "It's that type of picture that will lead to an understanding of why these compounds cause polymerases to make mistakes," she says.

Indeed, while this latest polymerase photo exhibition offers a great example of basic research, exploring the activity of these viral or bacterial enzymes may not be that far removed from a medical use, scientists stress.

"Polymerases are therapeutic targets," notes Joyce. For example, AZT, one of the earliest effective HIV drugs, battles the AIDS virus by interfering with its DNA polymerase, a protein called reverse transcriptase.

A more detailed understanding of the structure and function of polymerases in general, says Joyce, might therefore enable researchers to develop improved HIV treatments or even generate a new line of antibiotics. □