Watching DNA at Work

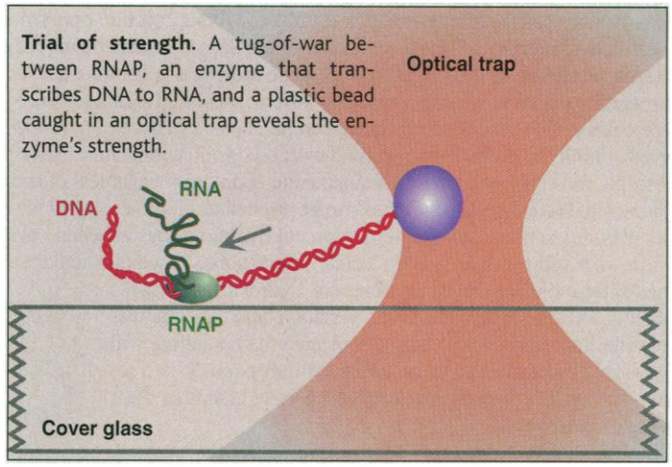
Optical traps and similar tools are allowing researchers to get a close look at a single strand of DNA and its workmate proteins when they are engaged in the business of replication and gene expression

Few biomolecules can claim to be as well studied as DNA. Ever since James Watson and Francis Crick unraveled the structure of the double helix at Cambridge University in 1953, researchers have spent billions of dollars and untold sleepless nights trying to understand and manipulate the molecule of life. They've sequenced entire genomes for more than 20 organisms, and the human sequence is expected to succumb in the next few years. But in virtually all these studies, researchers examine the collective, herdlike behavior of many thousands of copies of particular DNA fragments. Now, in a handful of biophysics labs around the world, a quiet revolution is under way: Researchers are getting a fix on the behavior of single DNA strands.

Just as an ecologist uses radio collars to track the movements of individual animals, these researchers are using tools such as lasers and magnets to gain a wealth of new insights into how DNA twists, turns, and stretches. In the very latest work in this rapidly emerging field, investigators are going beyond the simple mechanics of the DNA molecule to get an intimate look at the way different proteins work to cut, copy, and splice it. Such studies can reveal, for example, the precise force that a single protein-based motor exerts as it crawls along a DNA strand translating the code into RNA. These intricate observations even show that at some points the motor works fast and efficiently, and at other times it stalls out.

"By looking at single molecules, you can answer questions you can't possibly address with the broad-brushed approach of traditional biochemistry," says Princeton University biophysicist Steven Block. Such studies, Block adds, "have opened up these whole new areas of micromolecular mechanics," the study of molecule-sized machines. By engineering the genes for these protein machines, researchers will be able to see how alterations to their structures affect their operation. This will "allow you to study the molecular mechanisms that lead to motion," says Block. Adds Jeff Gelles, a biochemist at Brandeis University in Waltham, Massachusetts, "I think that virtually all the DNA enzymes will be studied in this manner."

DNA and its companions aren't the only molecules being pursued by single-molecule researchers. Chemists are using the approach to track changes to a variety of individual molecules (see the following articles). But DNA and its workmate proteins are generating interest in part because of DNA's central role as the cell's database, as well as more practical considerations. Among them is DNA's titanic size (the largest human chromosomes span a whopping 9 centimeters when fully extended), and its rugged nature. "DNA is a very robust molecule to work with, so it's a good one to start on," says Vincent Crouquette, a biophysicist at the Ecole Normale Supérieure (ENS) in Paris.



Trial of strength. A tug-of-war between RNAP, an enzyme that transcribes DNA to RNA, and a plastic bead caught in an optical trap reveals the enzyme's strength.

The key to manipulating DNA was the development of optical traps, which use tightly focused laser beams to snag tiny plastic spheres and other particles, as well as related techniques for manipulating tiny objects. By linking spheres to either end of a DNA strand and manipulating the laser beams, researchers can test the mechanics of the molecule, in essence seeing how much force DNA can withstand when stretched and twisted. Through the mid 1990s, teams found that the molecule, when pulled apart at the ends, initially stretches like a rubber band. Pull harder and the double helix actually unwinds somewhat, stretching the strand even further before breaking altogether. While such knowledge may seem arcane, it's turning out that DNA's mechanical properties are critical to the way some enzymes work with it.

In one recent study, for example, Crouquette, David Bensimon, and their ENS colleagues revealed the mechanical details of a new type of coiling pattern that is thought to occur when DNA is transcribed to RNA in the cell nucleus. The researchers attached one end of a DNA strand to a magnetic bead and the other to a glass slide. Then they applied a magnetic field to rotate the bead, twisting the helical strand so that it coiled back on itself, similar to the way a helically coiled telephone cord can become twisted. When they altered the magnetic field to pull back on the bead, they found that only a small applied force creates a new type of structure—essentially turning the DNA helix inside out—that has previously been shown to occur when the enzyme RNA polymerase (RNAP) transcribes DNA into RNA. This suggests—although it has yet to be confirmed—that the enzyme induces the awkward twist to copy a DNA strand more easily.

Single-molecule studies are leading to other insights into the way RNAP manipulates DNA. In the 30 October 1998 issue of *Science*, for example, a team led by Michelle Wang, a former postdoc in Block's lab at Princeton, reported that they had managed to gauge the force that a single RNAP molecule can exert as it crawls along a DNA strand, churning out an RNA version of the DNA code in the process. To do so, the team "set up the world's tiniest tug-of-war and followed it," says Block.

First they anchored an RNAP molecule to a glass slide. They then connected one end of a piece of double-stranded DNA to a plastic bead, which they could move back and forth with a laser-based optical tweezer, and fed the other end to the RNAP. The enzyme normally walks along the DNA strand, unzipping the pair of single strands in the double helix, reading the DNA code from one strand, and assembling RNA versions of each base pair. In this case, however, because the RNAP was fixed in place, it was forced to pull the DNA past it like a sailor hauling in a jib sheet. When the strand was pulled taut, the researchers were able to tug on the bead and determine that RNAP can pull with a whopping 25 piconewtons of force—four times that exerted by myosin,

CREDIT: WANG ET AL., *SCIENCE* **282**, 902 (1998)

the protein that contracts muscles. "RNA polymerase is the most powerful mechanoenzyme yet studied," says Block.

But it's not the only one coming under close scrutiny. At the Biophysical Society meeting last month in Baltimore, Maryland, Block's postdoc Thomas Perkins reported that the Princeton team had succeeded in performing an experiment similar to their "RNAP tug-of-war" with a completely different type of motor protein called lambda exonuclease. This enzyme—part of a bacteria-infecting virus—chews up one of DNA's double-helical strands, leaving behind just a single strand.

In this case, when the researchers set up their tiny force meter they found that the motor was capable of exerting at least 5 piconewtons of force. However, unlike RNAP and other molecular motors which are powered by the ubiquitous cellular fuel adenosine triphosphate, lambda exonuclease is fueled directly by the energy liberated in DNA's broken bonds. The new experiment, says Perkins, now allows researchers to explore just how many DNA bases lambda exonuclease can clip in one stretch and whether this occurs in a steady or sporadic fashion, information that is likely to be crucial for researchers working to sequence single strands of DNA (see sidebar).

Still other examples of fantastic mini-motors that manipulate DNA continue to emerge nearly every month. At the University of California, Berkeley, for example, Carlos Bustamante and his colleagues are using single-molecule techniques to study the complex way in which the cell manages to pack up to a meter's worth of DNA into its nucleus, just a few millionths of a meter across. At the heart of this packing process are chromatins, assemblies of DNA wrapped around a series of proteins. "The structure of chromatin is of central importance to gene expression," says Bustamante, because that structure determines which genes are readily accessible to other proteins called transcription factors that turn on gene expression. So Bustamante's team is pushing and pulling on single chromatins in an effort to learn about the forces that hold these collections together.

Meanwhile, at the ENS, Bensimon, Crouquette, and their colleagues are unraveling the mysteries behind an enzyme called topoisomerase that itself unties knots that form in DNA as it is unpacked in the nucleus and copied during cell division. Understanding how this motor works, says Bensimon, could prompt the development of novel cancer drugs capable of blocking the enzyme and thereby preventing cancer cells from replicating.

Even these examples are just the beginning. "It's a field that is only just starting," says Bensimon. Groups around the globe are already gearing up to take a look at a wealth of other enzymes, such as transcription factors, as well as helicases and gyrases, which help pack and unpack DNA coils within the nucleus. With single-molecule biophysics, says Bensimon, "there is a lot of molecular machinery in the cell that is now open to study."

–ROBERT F. SERVICE

Deconstructing DNA for Faster Sequencing

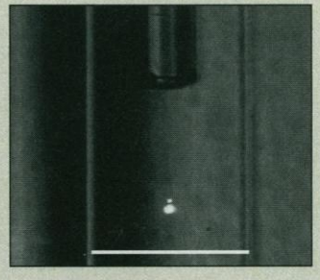
The international race to sequence the human genome has turned gene sequencing into a high-speed—and high-profile—endeavor. At its heart are machines that create thousands of copies of DNA fragments as a first step toward decoding the sequence, one nucleotide base pair at a time. But a handful of groups around the world are working to steal a bit of the sequencing limelight with new approaches that decode single copies of DNA. Because single-molecule sequencing has the potential to speed up the sequencing process, "[it] could become very important," says Jay Trautman, one of the technique's pioneers at the biotech company Praelux—formerly Seq—in Lawrenceville, New Jersey.

The push for the technology comes from the fact that current DNA decoding schemes sequence relatively short stretches of DNA, each about 1000 base pairs long. Researchers wanting to sequence a gene containing, say, 100,000 base pairs must sequence overlapping fragments of the gene and then use complex computer programs to reassemble the pieces in the right order. Single-molecule sequencers, in contrast, hope to sequence DNA segments as long as 50,000 base pairs, which would simplify and speed up the task of putting the puzzle back together.

The key to the technique's long sequencing lengths is a protein called an exonuclease, which degrades DNA by munching its way through tens of thousands of individual bases, one by one, like a molecular Pac Man. In theory, once each base is clipped off, researchers can channel it away and identify it with a laser-based detector.

But spying single bases isn't a simple task. The most common approach—being pursued by chemical physicist Richard Keller's team at Los Alamos National Laboratory and other groups at the Max Planck Institute for Biophysical Chemistry in Göttingen, Germany, and the Karolinska Institute in Stockholm, Sweden—is to initially tag each of the four types of bases in DNA with its own fluorescent compound. The researchers then fix one end of the DNA strand to a tiny plastic bead that is then held steady with a laser inside a tiny flow chamber. An exonuclease on the other end of the DNA clips off bases from one of DNA's two strands, which then flow past another laser, revealing their presence with a bright flash.

At the Photonics West meeting in San Jose, California, in January, Keller's team reported successfully detecting and identifying individual labeled nucleotides cleaved from DNA.



Decoder. DNA held by one laser (small dot) is sequenced by another (large dot).

But for now they're still plagued by false-positive signals: The fluorescent dye compounds stick to the beads when the DNA strands are attached and later wash past the laser at a rate of about three per second. The exonuclease, meanwhile, only clips one to five bases off the strand every second. "We're optimistic" that the technique will eventually pan out, says Keller. "But we're not there yet."

In search of faster sequencing speeds, Trautman and his colleagues are trying to accomplish the job on "native" DNA. Without tags in the way, exonuclease can clip about 12 bases a second, says Tom Perkins, Trautman's collaborator at Princeton University. But it's trickier to detect untagged bases. Though still in the early stages, the Praelux team is working on a way to repeatedly drag a DNA strand across a surface, clip bases off with the exonuclease, and fix them to the surface where they can be chemically modified for easy identification.

Even if the novel sequencing approaches don't pan out quickly, single-molecule DNA technologies are still likely to bear fruit. The technique, for example, has already shown promise for DNA fingerprinting, which could speed forensic testing in the field and help detect potential bioweapons threats. One way or another, expect to see single-molecule DNA studies grabbing a little limelight.

–R.F.S.

CREDIT: WERNER *ET AL., PROCEEDINGS OF SPIE*, IV, 3602 (1999)