Getting Physical with DNA

Stretching, twisting, prodding, and packing molecular strands

By IVARS PETERSON

Molecules of DNA are essential to life on Earth. They carry the genetic code that regulates the construction of proteins, which in turn orchestrate a host of biological processes and provide the basic structure of cells.

A single DNA molecule may stretch many millimeters in length. Crammed into a cell nucleus only a few micrometers wide, DNA must fold and coil itself into a remarkably compact configuration without getting tangled and without losing its ability to replicate and to perform other vital functions.

The molecule itself consists of two strings of nucleotides that are joined to form a ladder, then twisted into a right-handed helix. In solution, DNA behaves like a long, braided thread. Buffeted by molecules in the solvent, it bends in random directions and often ends up in a loose coil resembling the twisted cord of a telephone receiver.

Portions of DNA assume different configurations under different conditions, depending on their activity. During transcription, for instance, an enzyme straddles a DNA strand, untwisting and stretching a section of the molecule to expose the nucleotides and begin the process of constructing shorter strands called messenger RNA (SN: 3/29/97, p. 188).

To master the details of transcription, scientists must first understand the forces an enzyme exerts on DNA to uncoil and stretch the molecule, then separate its complementary strands. Researchers are therefore treating DNA molecules as minuscule springs or rods and are measuring such characteristics as their elasticity or stiffness.

"What's new now is our ability to do biophysics with individual molecules," says physicist Paul K. Hansma of the University of California, Santa Barbara.

Researchers can use light beams—laser tweezers—to tack down one end of a molecule, grab the other end, and pull on it. With an atomic force microscope, they can not only obtain images of biological molecules sprawled on a surface but also measure the forces involved in folding or stretching these strands.

"We can act on a single molecule mechanically and see its response," says Carlos Bustamante of the University of Oregon in Eugene. "A few years ago, that was quite impossible."

Such single-molecule studies provide insights into the physical basis of fundamental biological processes and contribute to the development of improved methods of separating, purifying, and analyzing DNA (SN: 3/8/97, p. 144), an essential part of modern genetics. DNA molecules themselves have distinctive characteristics that might make them useful as components of advanced, high-speed computers (SN: 7/13/96, p. 26) and as the building blocks of novel materials.

A number of recent advances in the biophysics of DNA were highlighted in March at an American Physical Society meeting in Kansas City, Mo.

Whether found in a mattress or a ballpoint pen, a spring is essentially a piece of stiff wire coiled into a helix. The force that such a spring exerts as it is compressed or stretched results from a complicated pattern of electromagnetic forces among the atoms making up the wire.

Nonetheless, a spring taken as a unit has measurable characteristics and responds in a particular, predictable way to an applied force. In many situations, for example, that force is roughly proportional to the distance to which a spring is extended.

Gently stretching a DNA molecule in solution reveals that for small extensions, it behaves much as an ordinary metal spring does and has a characteristic stiffness, or spring constant. Moreover, longer DNA molecules deform more easily than shorter ones, just as one would expect for metal springs.

Calculations based on theory indicate that the force it takes to stretch a DNA molecule to almost its full length is comparable to the drag acting on a micrometer-size object as it moves through the fluid inside a cell. This means that DNA is highly susceptible to changes in configuration in its natural environment.

Bustamante and his coworkers have measured the elasticity of a single DNA molecule. In their initial set of experiments, the researchers chemically attached one end of a DNA molecule to a glass slide and fastened a magnetic bead 3 micrometers in diameter to the other end. They could then stretch the DNA by applying a magnetic field and measure the extension by observing the position of the bead under an optical microscope.

The results suggest that when a modest force is applied to the molecule in solution, DNA acts like a braided thread of a certain stiffness writhing through three-dimensional space.

In more recent experiments, Bustamante and his colleagues have investigated the behavior of a single DNA molecule at higher forces, when the strand no longer behaves like an ideal spring. This time, the researchers attached a bead to each end of a DNA molecule, with one end held fast by suction and the other slowly pulled away using laser tweezers.

"The molecules display a number of novel and interesting features," Bustamante says. By pulling hard enough, it's possible to straighten the molecule; pulling even harder causes the molecular backbone itself to stretch, straining the chemical bonds holding the chain together.

In one study, the researchers found that a nearly constant force of about 65 piconewtons (pN) stretched DNA well past its normal length in solution. After nearly doubling that length, the force required to continue extending the molecule abruptly increased to 80 pN. When the tension was released, the overstretched DNA snapped back to its original state.

Theoretical models suggest that DNA undergoes a reversible transition to a new stretched form. If the molecule is free to rotate as it is stretched, the double helix unwinds into a ladder configuration, leaving the two backbone strands lying parallel to each other. If the rotation is blocked, the paired nucleotides (rungs of the ladder) get tilted at an extreme angle, producing a longer, thinner molecule.

An enzyme may bring about such stretching to expose the nucleotides of a DNA molecule, making it easier to gain access to the information contained in the genetic code.

Gil Lee and his colleagues at the Naval Research Laboratory in Washington, D.C., have used an atomic force microscope to measure the force between the complementary strands making up the double helix of DNA.

Normally, an atomic force microscope's needle tip, hanging down from a movable

cantilever that resembles a miniature springboard, rides back and forth across a surface. Measurements of the vertical deflection of the cantilever provide a contour map of the surface and any molecules pinned to it.

To measure the force associated with forming a DNA double helix, Lee and his team first coated the tip and the surface with short molecules from single strands of DNA. They then introduced longer, complementary strands into the solution between the tip and the surface.

When the coated tip and surface were brought close together, the longer strands would sometimes bind to the shorter strands and bridge the gap. The researchers could then measure the force required to pull the tip and surface apart and eventually rupture the bonds between the complementary DNA strands.

The results indicate that the complementary strands can be moved apart a surprisingly large distance with relatively little force before the bonds finally break and the microscope's tip snaps away from contact, Lee says.

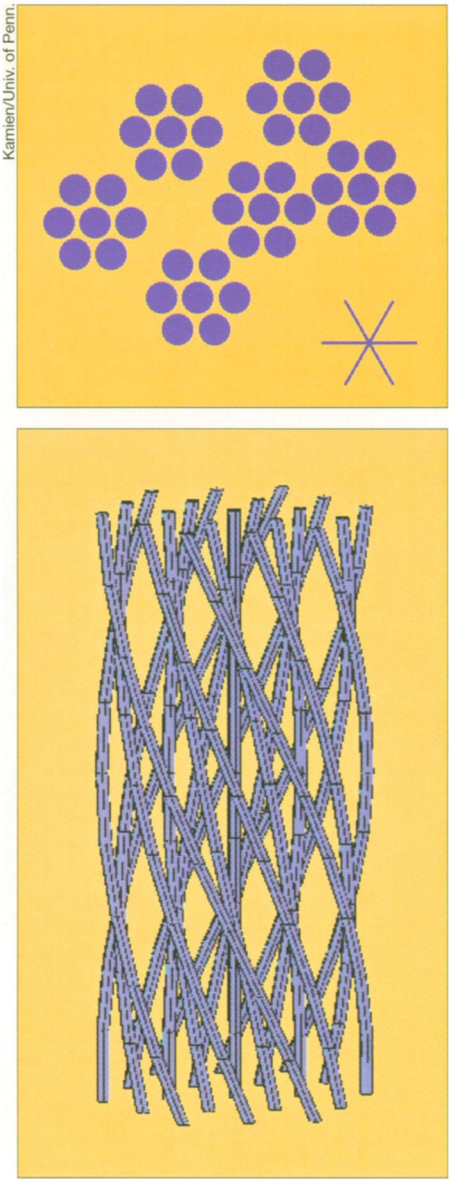
The same technique can be applied to other pairs of biological molecules that attract each other. The technology may eventually lead to highly sensitive methods for studying molecular adhesion, Lee notes, whether the adherence of barnacles to ship surfaces or the interaction between an antibody and its antigen.

In many instances, lengthy DNA strands must pack themselves into an extremely small space, for example, the head of a sperm cell (SN: 3/22/97, p. 172). Similarly, a virus typically crams its cargo of nucleic acids into a minuscule, tight-fitting protein shell (SN: 3/25/95, p. 186).

The intrinsic stiffness of DNA molecules makes them behave somewhat like long, wiggly rods when crowded together in solution. Such rods tend to align themselves to form orderly arrangements characteristic of a state of matter known as a liquid crystal.

Helmut H. Strey and his coworkers at the National Institutes of Health in Bethesda, Md., have studied liquid crystals made of biological polymers, focusing on the interactions between DNA molecules in a crowded environment. "DNA is especially well-suited for studying these polymer liquid crystals because DNA molecules can be easily tailored and manipulated with modern biochemical and molecular biological techniques," Strey says.

For example, the researchers can easily prepare solutions containing DNA molecules of the same length, ranging from a few nanometers to a centimeter. They can then measure the amount of energy it takes to push DNA molecules together to form a liquid crystal and determine how much the molecules repel each other.



Kamien/Univ. of Penn.

Crowded together, DNA molecules can arrange themselves so that the strands lie in roughly the same direction. A cross section (top) at right angles to the strands reveals that the molecules (circles) tend to cluster into hexagonal groups with a characteristic orientation. The clustered molecules may then twist along the DNA axis, maintaining the hexagonal clumps but changing their orientation (bottom).

Strey and his colleagues have prepared solutions of a given length of DNA that contain different concentrations of DNA and have used X rays to probe the structure of the resulting liquid crystals. At very high concentrations, they discovered a liquid crystalline phase never before observed in any material.

In this phase, DNA molecules are lined up nearly parallel to each other, like a forest of tall pines. Starting at one strand and going at right angles to it in any of six well-defined directions, as if along a spoke of a wheel, brings one to another strand. However, unlike the situation in a hexagonal arrangement in a true crystal, the distance to an adjacent strand in a given direction isn't fixed. "You don't know how far you must go [to get to the next strand]," Strey notes.

The right-handedness of the DNA double helix is expected to induce some form of twisting in the strands of the liquid crystal that is analogous to the way a twisted-up phone cord tends to coil around itself.

Physicists Randall D. Kamien of the University of Pennsylvania in Philadelphia and David R. Nelson of Harvard University propose the existence of a novel, braided liquid crystal phase, in which the long DNA molecules lie in the same direction and a slice at right angles through the sample would reveal the six directions in which neighboring strands lie. As one moves along the DNA axis and looks at different slices, these directions shift, depending upon the degree of twisting shown by the molecules.

"Since biological materials can be manipulated via genetic engineering, this opens up the possibility of creating new liquid crystalline phases," Kamien says. Such phases could prove useful as the basis for fast, high-resolution electronic displays and other devices.

Interestingly, the braided liquid crystal phase proposed for DNA may also arise in superconductors in high magnetic fields. When the magnetic fields are strong enough, they create an array of vortex lines around which electric currents swirl (SN: 11/27/93, p. 358). Forcing a current through the material in the same direction as the magnetic field could produce a braided vortex configuration similar to the liquid crystal arrangement predicted for DNA, says Nelson.

During the last few years, researchers have used single-molecule techniques to characterize other biological molecules as well. For example, they have studied changes in the shape of an enzyme in action, the opening and closing of channels in membranes, the operation of molecular motors (SN: 3/22/97, p. 173), and the unfolding of protein molecules.

"The ability to treat single molecules as if they were just springs or rubber bands in a freshman physics lab is just incredible," says Pennsylvania's Philip Nelson. "We can manipulate molecules as if they were macroscopic objects."

"It's a really exciting time," Bustamante comments. And there's still room for considerable improvement in the instrumentation, observes Hansma. Right now, researchers can study individual molecules in solution. Eventually, Bustamante predicts, it may be possible to use laser beams to manipulate molecules inside cells.

Says Hansma, "We're looking at the forces involved in life processes." □