Dancing DNA

Tripping the light fantastic with heredity's master molecules

His work will never win an Emmy, but Japanese biophysicist Mitsuhiro Yanagida nevertheless made television history a decade ago when he captured DNA in a crude black-and-white action video. Borrowing the concepts and camera technology used by astronomers, the Kyoto University scientist became the first to film individual DNA molecules — live. The footage attracted little attention at the time, and Yanagida eventually moved on to other work.

Now several U.S. scientists have adopted his approach to witness the dance of individual DNA molecules as they twirl and snake through gels or bend and stretch in solution. The researchers' success in solving a long-standing mystery about how gels can sort DNA molecules by size convinces them that this imaging technique — which uses fluorescence microscopy — will revolutionize molecular biology and materials science.

"We're developing single-molecule methodologies for doing molecular biology," says David C. Schwartz, a biophysicist at New York University in New York City. "The field is going to break wide open."

Through the eyepiece of his microscope, and then at his computer screen, Schwartz plans to pinpoint genes on chromosomes and study the interactions between DNA and certain proteins. Carlos Bustamante and Steven B. Smith at the University of Oregon in Eugene use a similar system to investigate how DNA and chromatin — chromosomal DNA tightly bound to histone and other proteins — fold into their functional forms. The Oregon researchers are now testing the mechanical properties of these molecules, and they anticipate the day when they can manipulate the molecules as well as watch them.

Chemists have begun eyeing the technique as a means to improve their understanding of the dynamics of polymers and other long, complex molecules. Even the Department of Defense expresses interest: Bustamante may get some stunning footage of polymers quelling turbulence later this year, which could help Navy scientists develop techniques for reducing friction on its vessels.

"The idea that you can see the molecules and how they behave is very important," says Bustamante.

While certainly of scientific interest, the images may also offer technological insights. Studies of protein folding and of DNA movements could speed the development of useful protein-based products and of new purification procedures for biotechnology, Bustamante suspects. Research into polymer dynamics may lead to more versatile plastics, or perhaps to new polymer lubricants.

The imaging technique defies the conventional wisdom that optical microscopes cannot resolve something as tiny as a molecule. "We play a trick," Bustamante admits. The secret lies in the design of the experiment and in the length of the DNA.

The technique involves attaching a fluorescent dye to DNA molecules to form a complex that glows. "What you actually see is the dye," explains Yuqiu Jiang, a graduate student working with Bustamante. Light shining down through the microscope causes the dye in the sample to fluoresce, and a filter blocks any stray light that might otherwise reflect back through the eyepiece.

Thus, just as one can watch very distant stars inch across a dark sky, these researchers can track the glowing DNA as it moves against a dark background. Indeed, the molecules look a little like shooting stars: Their leading ends shine brighter than their trailing tails. The researchers cannot resolve the molecule itself in much detail, but they can follow its movements.

"That's the beauty of it," Schwartz says. "You can watch the molecules and see what is going on."

"We can basically do chemistry with a single DNA molecule," Bustamante says. He has built a tiny experimental chamber for this work. He puts a molecule inside and magnifies it—first through the microscope's optics, then many times more as he blows up the image on a TV monitor. The squiggle that swims across the screen appears more than 5,000 times its original size.

Getting a good image, however, has proved quite challenging, Bustamante reports in the 1991 *Annual Review of Biophysics and Biophysical Chemistry*. Through experimentation, he learned how much dye to use. Too little dye resulted in a faint image, but too much dye in the sample created light pollution, making the DNA specks difficult to follow. Over time, he added an image intensifier that helps make weak picture signals clearer. He and his colleagues have also gleaned the importance of making sure their apparatus is properly grounded so that motors, lamps and other fixtures do not create spurious background signals.

Schwartz' team at NYU linked the camera that records their microscopic subjects directly to a computer, which digitizes and enhances the image.

Both groups initially used their microscope-video systems to study DNA's movement through electrophoretic gels. A standard technique for separating DNA pieces of different sizes, electrophoresis relies on an electric field

to gently draw charged DNA particles of varying sizes down a gel. Though molecular biologists depend on gels for electrophoresis, they never really understood how the technique worked.

Videos created by the Oregon and NYU groups revealed that DNA bunches up and stretches out like a caterpillar as it deforms to fit through the pores in the gel. If its brighter "head" cannot find a pore quickly enough, then the molecule contorts. The tail overtakes the head, hooks around and starts pulling the molecule as if wanting to take over as the leading end. This action impedes the particle's progress. "The molecule is fighting with itself," Bustamante says.

The larger the piece of DNA, the more difficulty it has maneuvering through pores and the slower its progress, says Schwartz. Thus, pieces sort themselves by size along the length of the gel.

The two teams have also studied a more complicated electrophoresis technique for sorting very large pieces of DNA. During this separation procedure, researchers repeatedly shift the direction of the electric field. With each shift, the charged DNA molecules try to change course. "The molecules almost have to turn a corner to follow the field," Bustamante explains. For a moment, the molecule tries to pull itself in several places at once, creating a bunch of small kinks. Eventually one kink prevails and leads the rest of the molecule sideways. Because bigger DNA pieces maneuver more slowly, they eventually begin lagging behind the smaller molecules passing through the gel.

By watching DNA dance its way through gels for the past four years, "we have [the principles behind DNA electrophoresis] about 90 percent figured out," says Schwartz.

Moreover, he thinks this work suggests that fluorescence microscopy may rival or surpass electrophoresis as a way of sizing DNA molecules. "I know what you can do with electrophoresis and I know what you can do with a microscope," says Schwartz, who helped develop one commonly used procedure called pulse-field electrophoresis. "By applying the basic principles of polymer physics," he adds, "you can find out a lot of things," such as a molecule's size. This microscope technique "is going to absolutely destroy gel electrophoresis," Schwartz asserts.

To expand the utility of the technique, his group is working to obtain more information from the DNA seen through the microscope, and Schwartz predicts that molecular biologists will now catch on quickly to the advantages of this approach. Looking at DNA under a microscope allows faster, more direct and less tedious analysis than using electrophoresis. "I think [the shift away from electrophoresis] is going to be dramatic," Schwartz says.

In the meantime, an ability to follow DNA in real time as it moves through gels or changes shape in different solutions offers other opportunities, Bustamante says. Such observations can help researchers assess the genetic material's behavior and understand how other molecules affect its shape and movement.

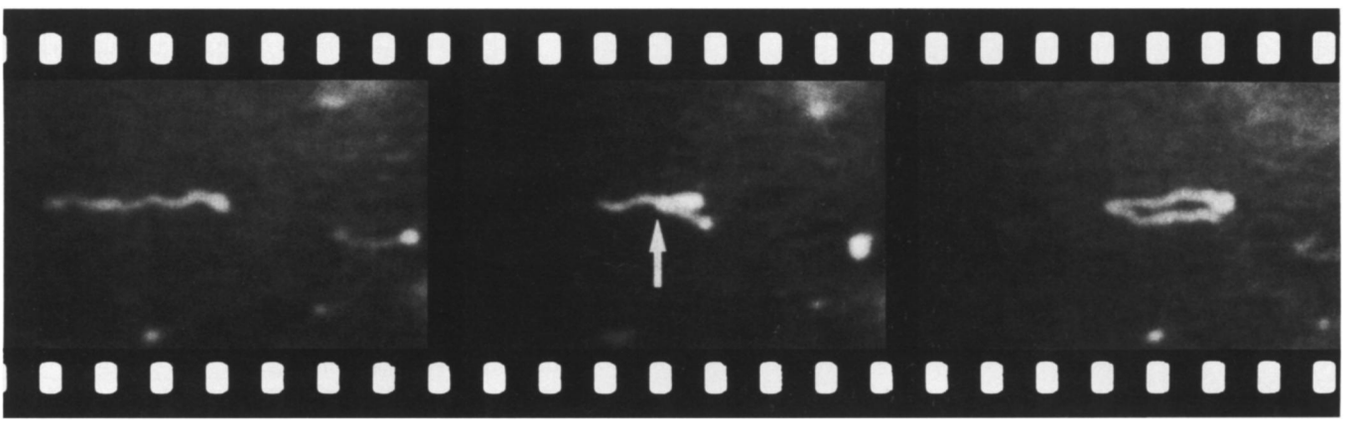
The capacity to study the dynamics of molecules in action is just what the Navy needs, says Ling-Siu Choi, a polymer chemist at the Naval Research Laboratory in Washington, D.C. Naval engineers know, for example, that small amounts of certain polymers, when dissolved in a solution or coating a ship propeller, can cut down on drag due to turbulence; but they are not sure how this happens. Before he and others can design a better anti-drag agent, Choi says, "we must see how [a new] molecule behaves in turbulent flow." He plans to work with Bustamante to learn more about these polymers.

In other experiments with glowing DNA, and more recently with chromatin, Bustamante's group has tested the molecules' physical properties. The researchers prompted one end of a DNA molecule to latch on to the cover slip that overlies the DNA sample during microscope viewing, and the other end to attach to a magnetic bead. Then they created a magnetic field that pulled the bead and straightened the DNA.

"It's like a Physics I experiment to measure the spring constant," Bustamante says. "It's a very clean and simple mechanical measurement."

As they vary the strength of the magnetic field, the Oregon biologists can measure corresponding variations in how far the DNA stretches. The ratio between the force of the field and the length of the DNA corresponds to the molecule's stiffness. Under different conditions, this property changes. For instance, Bustamante notes, "the DNA gets stiffer and stiffer as we lower the salt concentration [of the solution containing it]."

The team can also study torsion by twirling the magnetic bead to wind the DNA tight and then monitoring how long it takes to unwind under different conditions.



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Sequence shows single DNA molecules wriggling through a gel. When a molecule cannot immediately find a passage, it doubles up on itself, forming a hook (far right).

In the future, Bustamante hopes to alter and maneuver individual molecules while watching them on TV. Jiang hopes to harness the charged tip of a scanning tunneling microscope to nudge DNA around after first locating the molecules with fluorescence microscopy. In addition, Bustamante has noticed that he can dictate where the DNA moves by simply etching a shallow groove in the microscope slide, which the DNA seems to follow as it migrates on the slide. He now envisions molecular assembly lines, where a piece of DNA gets cut out and sent down a groove to react with an enzyme, and then moves on to a final site for continued observation.

This ability to direct molecules in a live-action video holds open the prospect "of manipulating molecules at a level that you've never been able to manipulate them before," Bustamante says. □