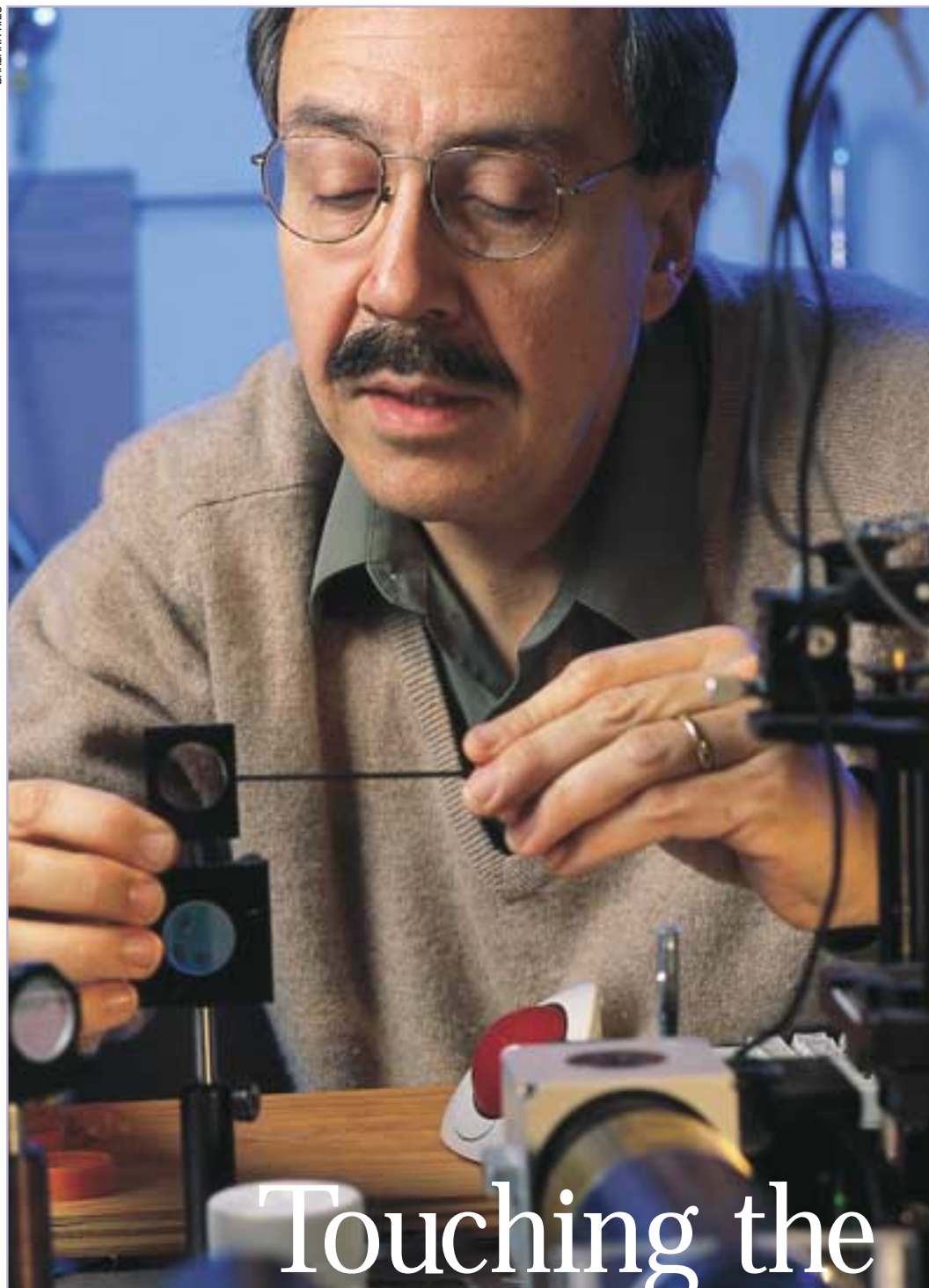


Scientific perceptions can sometimes shift so rapidly that it is difficult to remember an earlier reality. A decade ago, there were no topographical images of DNA in its natural, watery environment. What instead ruled the popular imagination, and to some extent the scientific one, was the double-helix icon and its elegant edifice of genes. This aesthetic view, even when elaborated by fluorescent staining images, said nothing about DNA's tensile properties and other physical properties, or about its ability to unwind prior to replication at speeds of more than 8,000 revolutions per minute.

Scientists knew, of course, that there must be physical forces at work, tiny parts and little motors that were working together to drive all the molecular movement essential to replication and transcription. But such forces were hidden and difficult to measure. Moreover, DNA molecules, although durable, were not unbreakable; they could not be probed and prodded by brute-force methods. To understand the mechanisms behind all the coiling, copying, snipping and splicing required a more subtle approach that did not interfere with the very activities researchers were trying to see and "feel."

Enter Carlos Bustamante, a self-described "patchwork biophysicist" with an insatiable curiosity about how biological machines work. In rapid succession in the early 1990s, his team produced both the first topographical images of DNA in water and the first measurements of DNA's elasticity. Still,

BARBARA RIES



Touching the

the images of these double-stranded helixes did little to explain something Bustamante had questioned in his earlier work: Just how elastic is DNA anyway, and why should anyone care?

The first question was fundamental, if unglamorous. It has long been thought that DNA must have a high degree of elasticity. After all, before replication the molecule wraps itself around nucleosomes—those structural supports made of proteins—packs itself and folds into the highly bent structure of chromosomes. With typical directness, Bustamante and his colleagues anchored one end of a DNA molecule to glass and attached a bead of known mass to the other. Gravity then did the rest. “It was crude,” he says. “But, for the first time, we were able to get some measurement of the force required to extend the molecule from its springlike resting state.”

Next, they tried magnetic beads. As reported in *Science* in November 1992, the beads moved in conjunction with external magnets, movement that Bustamante’s team was able to follow with a video camera. The researchers were also able to measure the force needed to stretch out, or extend, the single DNA molecule. “The fact that we could measure the elasticity was probably less important than the fact that we had opened the door to investigating and manipulating individual molecules,” he says. “Later, we learned that there was a difference in elasticity between single- and double-stranded DNA and that we could use this difference as the basis for new biochemical tests.”

Interesting, to be sure, from a purely physical point of view. But biologically significant? “It’s a question I used to hear a

lot, but not so much anymore,” says Bustamante, now a 49-year-old HHMI investigator and professor of biochemistry and molecular biology at the University of California, Berkeley. As the son of a physician, Bustamante understands the need to weigh the worth of research by its clinical potential. As a practical man, however, he realizes that such is not immediately obvious amid arcane mathematical equations.

Yet, he is not worried about his work’s

“I like to break things down to understand their controls and their parts and then put them back together—with, I hope, nothing left over.”

ultimate relevance. For apart from the intriguing discoveries his team has made about protein fatigue in muscle, Bustamante knows that deciphering how the molecular machines of the cell work will reveal deeper truths about biology.

“For example, we now know a lot of the details about how the parts of the machinery work during DNA replication, and we are learning how chemical energy is converted into movement and how efficient these motors are,” he says. “Once we have the machine, we can theorize and test why it is constructed that way.” The next step, he says, is to understand the relationship between these individual molecular machines and the many others that com-

bine to form the cellular factory.

If the goal is lofty, at its core it remains a mechanistic one—and not so different, at least in principle, from Bustamante’s childhood determination to take apart and rebuild toy cars. “It’s the same in biology,” he says. “I like to break things down to understand their controls and their parts and then put them back together—with, I hope, nothing left over.”

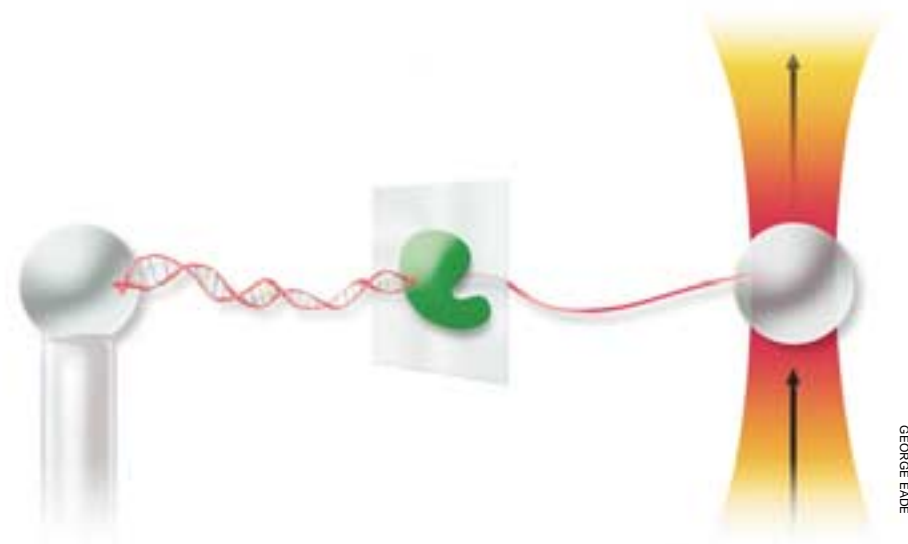
This need to reason and quantify reverberates throughout Bustamante’s life and career. At age 12, growing up in Peru, he built rockets and propelled them with the explosive combination of potassium perchlorate, sucrose and sulfuric acid. By age 16, he was working in his own home laboratory, studying the behavior of paramecia. By his mid-20s, he was an international Fulbright scholar at UC Berkeley with a background in mathematics and physics and a lasting love for microscopes. “Even as a young boy,” he says, “I was always trying to predict outcomes and to construct reasons for what I was seeing through the lens.”

If Bustamante’s instincts and interests seemed to make him an ideal candidate to study the structure and forces behind molecular activity, however, they were not a guarantee. “After I finished my scholarship at Berkeley, I wanted to go back to Peru and thought the best way to do that was to become more of a theorist,” he says. But challenged by his thesis adviser to build a machine that tested his theories of optical activity—a machine he built and used successfully—Bustamante stayed. And as improved technology and career opportunities converged in the 1980s, the curious biochemistry and physics student gradually

# INVISIBLE

## One Molecule at a Time

By Jeff Miller



GEORGE EADE

*This “optical trap” reveals the dynamics of DNA’s replication molecular machinery. Scientists use the device to measure the force that’s produced as DNA is converted from its single-stranded form (right) to its double-stranded form (left). Two beads hold the DNA in place. The bead on the left rests atop a pipette, while the bead on the right sits in a laser beam. The DNA stretches between the two beads, producing a measurable amount of force. When the polymerase molecule in the middle adds base pairs to the DNA, this force changes, causing the bead on the right to move slightly within the laser beam. Researchers can measure this movement optically. The more the bead moves, the more force the DNA produced. Knowing the amount of force given off at each moment helps scientists understand the “mechanics” of DNA synthesis. Adapted from Fig. 1a, Nature 404:103, 2000 © Macmillan Magazines Ltd.*

turned his sights to the biophysics of molecular movement and to its essential problem: How could you see, feel and measure what was going on?

Atomic force microscopy (AFM), invented by physicists in 1986, gave him both sight and insight. “AFM works by touching,” Bustamante says, “much like a blind person uses Braille.” In short, a cantilever with a sharp tip, often made of silicon nitride, is scanned over a surface at a constant force or height. The soft physical contact causes the cantilever to bend to accommodate changes in topography, the result of a repulsive atomic force that arises between atoms in the tip and atoms in the sample. As the tip moves, feedback mechanisms employing lasers and photodetectors measure the difference in the

light reflected off the back of the cantilever to record and translate it into a three-dimensional image of the scanned surface. In the late 1980s, while at the University of New Mexico, Bustamante and his colleagues had been among the first to use new DNA fluorescence staining techniques and wire electrodes to induce and study molecular movement under the microscope—in real time. AFM offered better visuals, something he had proved with his eye-opening topographical images of DNA, but it had the potential to offer more.

Exploiting this potential required continuing improvements not only in AFM but in fluorescence staining, which uses special dyes to make parts of the cell nucleus visible under the microscope. It

also speeded the development of related techniques, such as optical tweezers, which use the conservation of light’s momentum to trap tiny objects and manipulate individual molecules. Indeed, it was the tweezers’ ability to capture, move and stretch molecules and measure tiny changes in their fluctuating environment that enabled Bustamante and his team to determine the difference in elasticity between single- and double-stranded DNA. The trapping action occurs when the tightly focused light of a single laser, sent through a microscope, captures the desired target. Moving the light moves the target, without any need to actually touch the biological sample. The change in momentum as light spills from the trap provides both the trapping force and a way to measure this force.

With such refined techniques at their disposal, Bustamante and his colleagues set about putting DNA fragments to various microscopy and trapping tests. In some cases, such as that reported last year in *Nature*, they have introduced the enzyme DNA polymerase to a single strand of DNA and “watched” and measured its motion as it helped the strand pair off with the proper bases and rebuild itself into double-stranded DNA. “It’s amazing,” Bustamante says. “You can measure the bursts of activity as the polymerase loads onto the chain, replicates and falls off. You can actually follow biochemical processes by a single molecule in real time.” At a catalysis rate of more than 100 bases per second, it does not take long for the rubber-band shape of single-stranded DNA to be transformed into the distinctive “garden hose” of its double-stranded cousin.

“Whenever we study these biochemical processes at the single-molecule level, we find that the molecules have a random, almost chaotic behavior that is far from the average, smooth picture we get when we study whole ensembles of the molecules by traditional ‘bulk’ methods,” Bustamante explains. “Inside the cell, many of these fundamental processes are carried out by only a few molecules at a

time. I believe we'll get a more realistic view of the cell's inner workings if we can follow the work of each molecule individually.”

In other experiments, Bustamante uses different polymerases to investigate how much of the transcription process is controlled by the dynamics of the enzymes translocating over the DNA, pairing new nucleotides with their proper bases in the matching RNA. And in still different experiments, his team has used laser tweezers to stretch and unfold the giant protein molecule known as titin, which is

essential to muscle function. Apart from learning the tension incorporated into its coiled shape, the researchers have found that repeated unfolding induces a kind of molecular fatigue. “The protein recovers and refolds after a few minutes,” he says. “We are now trying to find out the advantages of this delay.” The answer may ultimately offer some clues to forestalling heart failure and the effects of Duchenne's muscular dystrophy, two conditions where titin—and muscle contraction—are highly compromised.

With each new experiment, Bustamante

and his team are learning more about the many molecular machines and testing their own predictions about why they work in the manner they do. At the same time, they are tinkering with AFM in pursuit of the day when they can achieve nanometer-scale resolution of biological samples in liquid, a resolution now possible only if the desired samples are in a vacuum. As for maintaining his own high-yield enthusiasm, Bustamante does not worry. “Loving science is the engine for doing it. I am self-propelled.”

## ROLES *and* MODELS

Carlos Bustamante remembers the moment well. It was a summer morning in 1964, and he was going through some of his father's books. He noticed one that happened to be written by a Spanish scientist, Santiago Ramón y Cajal, who received the Nobel Prize in 1906 for his work in determining the structure of the nervous system.

“This was a major turning point in my life,” Bustamante says. “I had a sense of inadequacy because the only scientists I knew about until then had foreign names, which did not resonate to my Spanish ears.”

Ramón y Cajal certainly could not have anticipated his future as a role model for an inquisitive teenage boy in Peru, but it is a role that Bustamante has understood and accepted as he has built his own academic and research career. “I do not consider myself an activist, but I am aware of what listening to me may mean to Hispanic students,” says Bustamante, an HHMI investigator at the University of California, Berkeley. “Many times, they have come up to me after a lecture and expressed their joy in hearing science spoken about with a Spanish accent.”

When he was teaching at the University of New Mexico in the mid-1980s, Bustamante inspired a generation of native Spanish-speaking students, many of whom were the first in their families to attend college. To him, these students represented the first link in what might become a family chain of professional achievement. “In most cases,” he says, “the educational success of one child had a positive effect on the other children in the family, who then sought some sort of professional degree or certificate of their own.”

Nor is Bustamante's symbolic significance limited to Hispanic students.

Trained in both physics and biochemistry, he has carved out as distinctive a research career as curiosity, opportunity and technology allowed. “There was no clear pathway for me,” he says. “I came to biophysics haphazardly.” Now, in this “new era of biophysics,” he advises nine biophysics graduate students in his laboratories in the physics and biology departments at UC Berkeley and has helped design a curriculum that exposes physics majors to biology and vice versa, with mathematics courses as the common bond.

Says Bustamante with confidence, “These students are becoming the phenotype for the biophysicist of the future, the ones who will deal at a completely quantitative level with biological problems.” Moreover, these future scientists could be the vanguard in a transformation of biology from an organic to a synthetic science. “It's clear,” he explains, “that in time we will stop studying what is and start building what is not.”

In the meantime, Bustamante continues to hone his own teaching techniques. “I always have been in love with learning,” he says, “so when I first started teaching, I assumed everyone else had the same feeling. I eventually learned that many people are afraid, so I think it is important to offer equal parts of enthusiasm and reassurance.”



Santiago Ramón y Cajal

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