STRETCH GENES

By unraveling individual DNA molecules under the microscope, physicists are learning about the elastic properties that are important to DNA's function.

Robert H. Austin, James P. Brody, Edward C. Cox, Thomas Duke and Wayne Volkmuth

Polymers—macromolecules composed of many small molecules—are vital to life. For example, proteins provide the cell with structure and carry out its mechanical and enzymatic work. Fatty acids store energy in their bonds, and lipids form the membranes that seal off the cell's aqueous interior from its aqueous exterior. Heredity lies in the very long polymeric strands of deoxyribonucleic acid—DNA.

The amount of DNA in a cell is mind-boggling. (See figure 1a.) There is enough DNA in one human cell to encode the approximately 100 000 genes in the human genome, and then some. This DNA is partitioned into 46 separate linear molecules, called chromosomes, 23 from each parent. All of it is crammed into the cell's nucleus, a compartment whose width is on the order of a few micrometers. It seems difficult to believe that this much material can fit in such a small space—much less replicate faithfully and fulfill all of its other functions—without posing enormous physical and topological problems. It fits because of the DNA molecule's conformation, the way the molecule is folded and twisted. (See figure 1b.)

DNA is a polymer of nucleotides, and human DNA contains two such strands of polymerized nucleotides (see figure 2), twisted into a right-handed helix. This form of DNA (referred to as B-DNA) was described by James Watson and Francis Crick in 1953 and is the conformation it takes in solution and, presumably, one of the conformations DNA assumes inside the cell.

DNA, like many other macromolecules, obeys Louis Henri Sullivan's architectural dictum that "form ever follows function." Under different physical and physiological conditions, DNA assumes many different configurations. When relatively little water is available to it, for example, the DNA molecule becomes shorter and squatter than B-DNA and takes on a conformation called A-DNA. During both replication and the first step of gene expression, when the DNA is "transcribed" into RNA, the DNA molecule must be unwound. In the opposite instance, when DNA is inaccessible to replicative and transcriptional machinery, DNA is supercoiled. Biologists have even discovered a left-handed helical form, called Z-DNA. But the repertoire of this molecule is even more versatile than that.

New technologies make it possible to manipulate and study *single* molecules of DNA. Using these techniques, which we will describe, can answer questions unapproachable by other techniques, such as the existence of new conformations. Single-molecule studies can also lead to practical insights with implications for the development of new laboratory techniques for analyzing, fractionating and sequencing DNA.

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The random coil

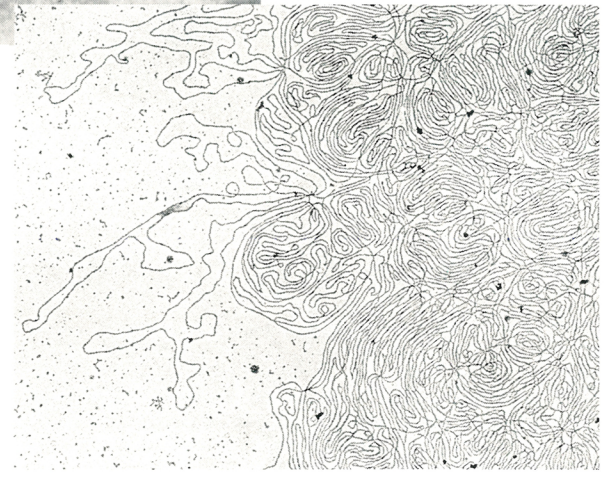
How can the equilibrium shape of a DNA molecule in aqueous solution be described theoretically? As is evident from figure 1b, the typical molecular configuration is thoroughly random, so a statistical approach is most appropriate. A simple observation about DNA's structure provides a clue about how to construct a model. Just as plaiting a pair of flexible threads yields a more rigid braid, so the entwining of a DNA molecule's two helical strands confers considerable stiffness upon the molecule. This suggests that the physical properties of DNA may reasonably be modeled by treating it as a uniform elastic rod. In such a simplified description, just two physical parameters suffice to characterize a molecule: the elastic bending modulus κ and the total (unstrained) axial length L. Since the path that the polymer follows may be described as a continuum curve $\mathbf{r}(s)$ writhing through space, this model is known as the worm-like chain (WLC).[1] The local elastic energy is proportional to the square of the curvature, so that the total energy of the molecule is

$$U = \frac{1}{2}\int_0^L \kappa \left(\frac{\partial \mathbf{t}}{\partial s}\right)^2 ds \qquad (1)$$

where $\mathbf{t}(s) = \partial\mathbf{r}/\partial s$ is the unit tangent vector and s is the coordinate measured along the polymer contour.

Although the rigidity tries to keep the DNA straight, the thermal forces buffeting the molecule act to bend it in random directions. The molecule is constantly in motion, continually changing its conformation. Given the form of the energy functional in equation 1, it is straightforward to show that, at any instant, the correlation of the tangent vectors decreases exponentially with their distance apart along the contour. The interplay between Brownian agitation and rigidity, then, determines DNA's persistence length P, which is the length scale on which the directionality of the polymer is maintained:

a



b

FIGURE 1. HUMAN DNA. **a:** The world's largest ball of twine, made by Francis A. Johnson of (fittingly) Darwin, Minnesota, also serves as a scale model of how much DNA is stored in the human diploid genome: approximately 1 meter or 3×10^9 base pairs. **b:** Individual strands of DNA in a human chromosome. There are no free ends, and thus we conclude that each chromosome is one DNA molecule.

$$P = \frac{\kappa}{k_B T} \tag{2}$$

When one zooms in to scales shorter than P, the molecule appears straight. But looked at from a distance, the molecule bends this way and that, and appears to be randomly coiled.

For DNA in normal physiological conditions, $P \approx 50$ nm, which is considerably longer than the molecular diameter of 2.5 nm, justifying the assumption about uniform elasticity. But it is nevertheless very short compared to the lengths of chromosomal DNA molecules, which run, on the average, about 50 mm in length. Viewed under a microscope, these huge molecules look like random coils, and their ensemble of equilibrium configurations has the same statistics as a random walk. Accordingly the mean square distance between the ends of a molecule is proportional to its contour length:

$$\langle R^2 \rangle = 2PL \tag{3}$$

For chromosomal DNA, the coil size R is much shorter than the overall molecular length. As an example, a chromosome from a simple organism such as yeast, which might contain a million base pairs and measure 300 μm when fully stretched, forms a coil about 5 μm across when it is at equilibrium in aqueous solution.

One consequence of the random-coil structure is that the average density of a DNA molecule, when packed as a Gaussian coil, decreases with increasing molecular size. In many physiological situations, such loose packing would pose a problem, so the cells must use a battery of proteins to locally bend the DNA and wrap it up in an orderly fashion. Precisely how proteins act on the DNA to accomplish such complex processes is one of the mysteries that experiments on single molecules aim to penetrate.

Entropic elasticity

At equilibrium, in its randomly coiled conformation, DNA is not easy to examine under the microscope. Stretching it out provides a much clearer picture. One way to do that is to use micromanipulation tools, such as optical tweezers or microneedles, to grasp the molecule by its two ends. As the ends are moved apart, the molecule reacts

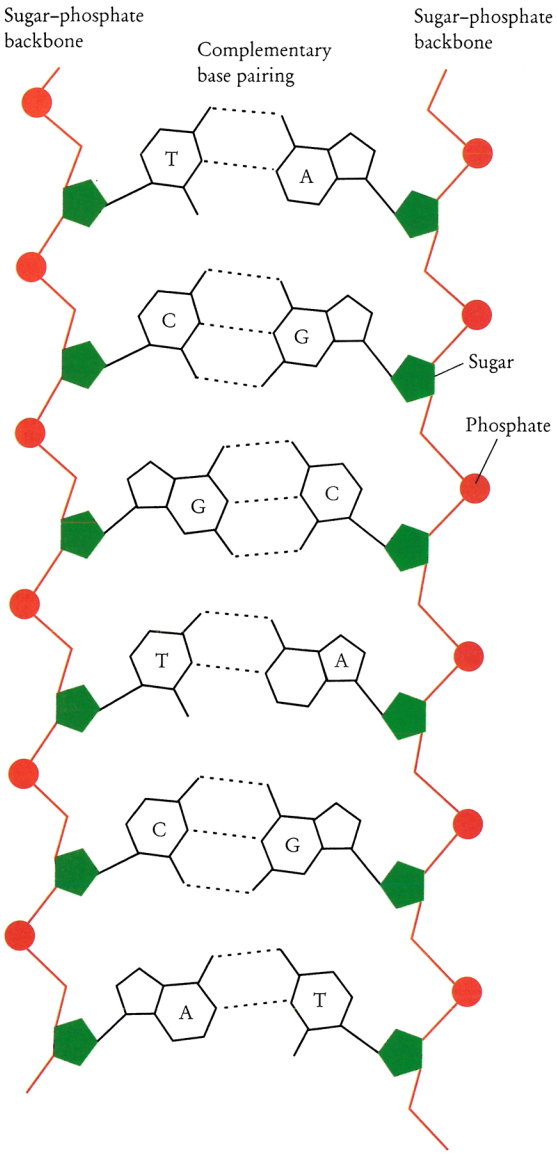


FIGURE 2. DNA IS A POLYMER of nucleotides, each of which consists of a nitrogenous base (A, T, G or C) attached to a phosphate–sugar backbone. The structure of double-stranded DNA resembles a ladder, in which two backbones form the side rails and the bases are hydrogen-bonded into pairs to form the rungs. The sequence of base pairs along the backbone is the genetic code. In its most common configuration, the DNA ladder is twisted into a right-handed helix, with each turn of the helix measuring 3.4 nm in length.

by exerting a force that resists the separation. This force is entropic in origin, ultimately a consequence of the Brownian impulses acting on the polymer that tend to keep it coiled. The force arises because the number of molecular configurations consistent with a given end-to-end vector **h** decreases as the magnitude of **h** increases. The full force–extension curve can be calculated numerically from the WLC model, but the general behavior is more easily understood by making approximations (see

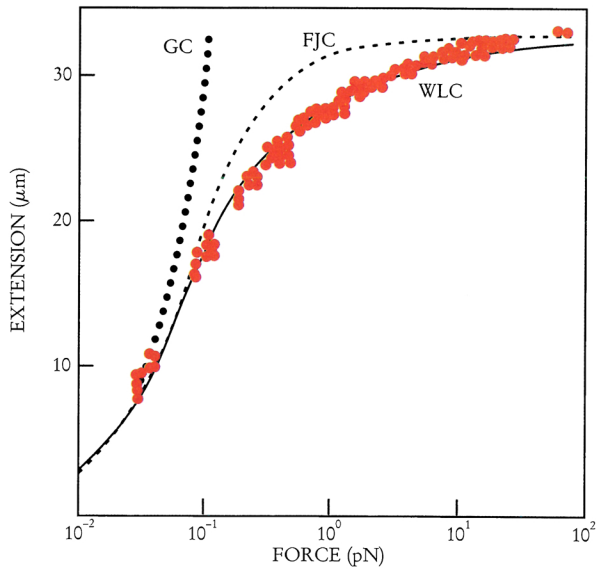


FIGURE 3. THE FORCE-EXTENSION CURVE of a single DNA molecule under traction applied at its ends. The data points from reference 3 are compared with the theoretical curves for the entropic elasticity of three polymer models: the Gaussian chain, the freely jointed chain and the worm-like chain, which fits the experimental data remarkably well.

the box on page 36).

A weak force perturbs the DNA molecule only slightly from its equilibrium shape. Consequently, the response of the polymer can be deduced from a knowledge of the equilibrium probability distribution of the end-to-end vector. Since the distribution is approximately Gaussian, this model is known as the Gaussian chain. A weak tensile force F applied at the ends of the molecule causes it to extend by a fraction of its contour length:

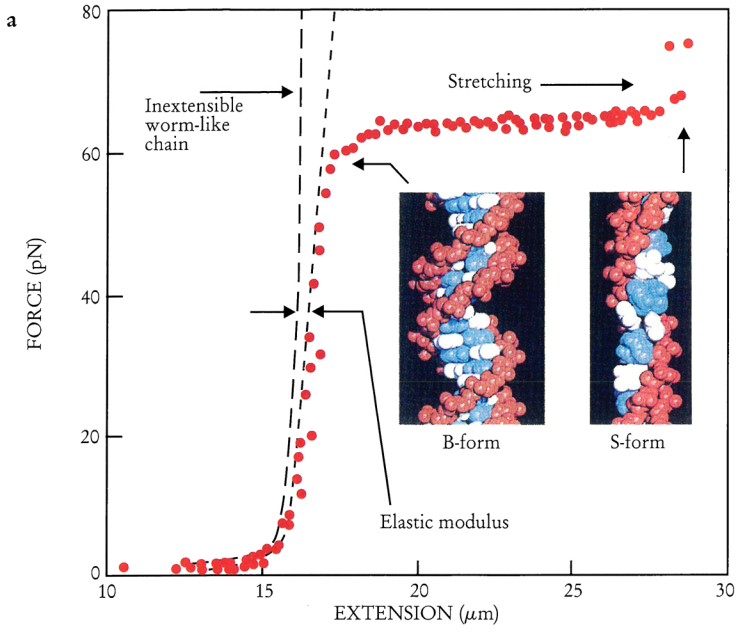
$$\frac{h}{L}=\frac{2}{3}\left(\frac{FP}{k_BT}\right), \quad h \ll L \tag{4}$$

This expression, which is valid for small extensions, is notable in a number of ways. First, the extension is linear in the force, so the DNA behaves as a Hookean spring with zero natural length. (That is, when **h** is zero, there is no deviation from a perfectly spherical Gaussian coil.) Second, longer DNA molecules deform more easily than shorter ones, just like macroscopic elastic springs. Unlike in everyday springs, however, the effective spring constant depends explicitly on the temperature—a signature of its entropic origin. Finally, the expression indicates that the force required to stretch out DNA to a significant fraction of its full contour length is as follows:

$$F_s \approx \frac{k_BT}{P} \tag{5}$$

In physiological conditions, $F_s \approx 0.1$ piconewton. This value is surprisingly small, weaker than the typical force generated by individual motor proteins, such as myosin or kinesin, and similar in magnitude to the typical drag forces acting on micrometer-sized objects as they are transported in the cell. Clearly, DNA is highly deformable in its natural environment.

When the applied force is stronger than F_s, the elasticity becomes nonlinear. It becomes harder and harder to stretch the DNA as it straightens out, and the end-to-end separation approaches the contour length, L. A ge-



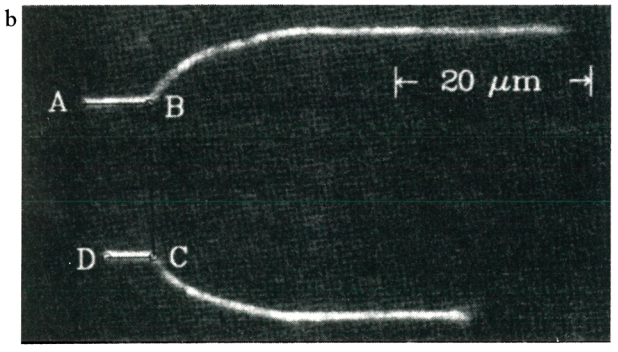


FIGURE 4. **a:** ELASTIC STRETCHING OF DNA beyond its natural length of 16.4 μm, and the subsequent structural transition to S-DNA, the hyperstretched form. The data points are taken from reference 5. The inset shows the structure of standard B-DNA and one of the predicted structures for S-DNA obtained by molecular modeling, taken from reference 4. **b:** A broken loop of DNA, stuck to a glass surface after the liquid that contained it passed to the left. The separation AD is greater than AB + CD, which indicates that this section of the DNA was overstretched in the meniscus just before it snapped. (From ref. 6.)

neric model of polymers that affords a straightforward picture of molecular stretching is the freely jointed chain (FJC). The polymer is represented as a chain of rigid rods connected by revolving pivots. When a force is applied to the ends of the chain, the Brownian swiveling of each rod is biased toward orientations that reduce its energy. Consequently, each rod acquires a mean orientation and the polymer as a whole becomes extended. In this model, the full force–extension curve can be calculated analytically. The Gaussian result is recovered at small deformations, but the contractive force diverges as the extension approaches the contour length.

In the WLC, the force diverges less strongly than in the FJC, since the polymer is able to bend continuously, rather than at a limited number of isolated points. As full extension is approached, the force–extension law has the form[2]:

$$\frac{h}{L} = 1 - \left(\frac{2FP}{k_B T}\right)^{-1/2}, \quad L - h \ll L \tag{6}$$

Measurements of DNA elasticity

How well does the actual conformation of the DNA molecule in solution agree with these theoretical considerations? To answer this question, Carlos Bustamante and his colleagues[3] made a direct mechanical measurement of the elasticity of a single DNA molecule. A fragment of viral DNA 97 kilobases long was chemically attached by one of its ends to a glass slide and by the other end to a tiny (3 μm) magnetic bead. Having created a DNA tether, it was relatively straightforward for them to stretch the DNA by applying a magnetic field and to measure the extension by observing the position of the bead under an optical microscope. The magnetic force was calibrated by comparison with the known value of the hydrodynamic drag on the bead in a flow.

The original publication[3] compared the extension curves to the FJC model and reported considerable discrepancies between experiment and theory, but the limitations of the FJC model were not widely recognized. Later publications done in collaboration with John Marko and Eric Siggia[2] reinterpreted the data in terms of the WLC. The agreement was found to be excellent, as shown in figure 3. This experiment with a single DNA molecule, then, has provided the strictest test of theories of entropic polymer elasticity to date. The WLC is evidently a remarkably good model for a stiff but flexible polymer.

Stretching DNA beyond its natural length

By pulling hard on the ends of a DNA molecule, it is possible to wring out all of the entropy and straighten the polymer. By pulling harder yet, might one stretch the molecular backbone, just as one can stretch a nylon thread? If so, the elasticity would correspond to the straining of chemical bonds along the DNA axis, and would therefore be of enthalpic, rather than entropic, origin. Using the WLC model, one can readily estimate how big a force is needed to observe such an effect. For a uniform elastic rod, the elastic constant for stretching (the Young's modulus E) is related to the bending modulus κ:

$$\kappa = \frac{\pi}{32} E d^4 \tag{7}$$

where d is the diameter of the rod. Thus, the Young's modulus of DNA can be calculated from the known values of the persistence length and the molecular diameter. Interestingly, the value obtained, $E \approx 10^8$ pascal, is comparable to that of a macroscopic sample of polyethylene. The force required to stretch DNA beyond its natural length by a factor of 10% is approximately 50 pN, and is independent of the molecule's length.

Two recent experiments undertaken to examine just what happens when DNA molecules are strained by forces of this magnitude have yielded startling results. Jean-Louis Viovy and his coworkers[4] used an ingenious setup in which the DNA was stretched between an optical fiber and a microbead. The fiber was chemically machined to have a low bending rigidity, so that it would be deflected by the tensile force in the DNA. The bead was pulled by a piezoelectric translator capable of precision displacements, and the deflection of the optical fiber was moni-

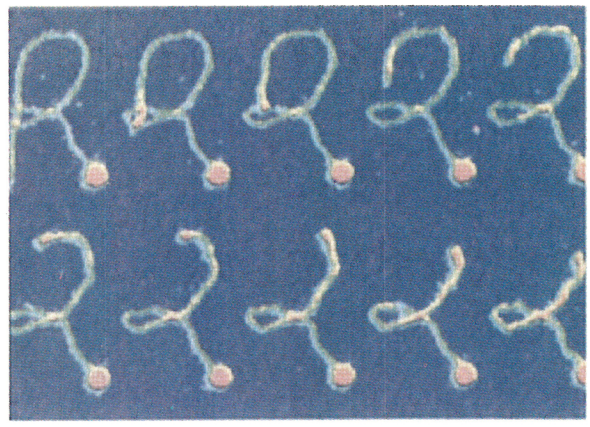


FIGURE 5. POLYMER REPTATION. A fluorescently marked DNA molecule is imaged after being dragged through a concentrated solution of other DNA molecules. It follows the letter "R" traced by the bead, providing evidence that the molecule's motion is confined to a tube, as described by the reptation model. (From ref. 11.)

tored by detecting the direction of the light emitted from its end. In the other experiment, Bustamante's group[5] attached beads to both ends of a DNA molecule; one was held fast by suction in the mouth of a pipette while the other was slowly moved away using an optical tweezer. Both groups found that the DNA could indeed be stretched to almost 110% of its solution length by applying a force of 50 pN.

But a surprise was in store for them when the force was increased yet higher. When the applied force was close to 70 pN, the DNA abruptly yielded and extended to almost twice its normal length (see figure 4). Subsequently, the force could be raised to 160 pN before the attachment between the molecule and bead eventually broke. This result is reminiscent of what happens when one plays with chewing gum, which is difficult to stretch at first, but beyond a certain point gives way easily. Unlike chewing gum, though, the overstretched DNA molecule reverts to its former state if the tension is relaxed.

In a receding meniscus experiment, similar to the one portrayed on the cover of this issue, David Bensimon's group[6] also observed a doubling of DNA's length when, by chance, both ends of the molecule were stuck to the surface. Eventually the DNA broke (figure 4b).

Molecular modeling[4] by Viovy's group indicates that the brusque deformation occurs because the DNA undergoes a reversible transition to a new form, which the group termed S-DNA (S for stretched). Moreover, it seems that the precise structure of S-DNA depends on the manner of pulling. If the molecule is free to rotate as it is stretched, the double helix unwinds to leave the two strands lying parallel to one another; the untwisted helix once again resembles a ladder in which the base pairs are the rungs. But if twisting is blocked, the double helix remains wound and the bases, which usually lie perpendicular to the axis of the helix, get tilted at an extreme angle. (See the inset to figure 4a.)

Astonishingly, the structural transition of DNA to a longer and thinner molecule had been anticipated many years previously by Maurice Wilkins and his coworkers.[7] In an experiment conducted even before the structure of DNA was established, they stretched a fibrous agglomerate of DNA molecules and observed the formation of a thin neck that could be reversibly extended to almost twice the original sample length. Moreover, the optical birefringence of the neck was of opposite sign to that of the unstrained region. In their 1951 article entitled "Nucleic Acids: An Extensible

Approximate Statistical Models of Polymer Chains

Gaussian Chain

A long DNA molecule at equilibrium in solution has random-walk statistics, so the probability distribution $p(\mathbf{h})$ of its end-to-end vector $\mathbf{h}$ is approximately Gaussian:

$$p(\mathbf{h}) = \left(\frac{3}{2\pi\langle R^2\rangle}\right)^{3/2} \exp\left(-\frac{3h^2}{2\langle R^2\rangle}\right) \tag{8}$$

If $\mathbf{h}$ is fixed, the configurational entropy of the DNA is $S = k_B \log p(\mathbf{h})$. Thus, when the ends are separated by h_z in the z dimension (but unconstrained in the other two dimensions), the polymer exerts a force

$$F = -T\frac{\partial S}{\partial h_z} = \frac{3}{2}\frac{k_B T h_z}{LP} \tag{9}$$

Inversion of this result gives equation 4. This model is clearly unrealistic at elevated forces since the polymer extends indefinitely as the tension in the chain increases.

Freely Jointed Chain

The freely jointed chain (FJC) model represents a polymer molecule as a chain of N rigid links, each free to swivel in any direction. Each link has length b, known as the Kuhn statistical length, and correspondence between the equilibrium configurations of the FJC and WLC is ensured by the choice $b = 2P$, where P is the persistence length. A force applied to the ends of the chain tends to align each link. The theory is familiar from the calculation of the orientation of a dipole moment in a uniform field. If a force F is applied to an FJC, the net projection of the polymer h_z along the direction of the applied force is

$$\frac{h_z}{L} = \mathcal{L}\left(\frac{Fb}{k_B T}\right) \tag{10}$$

where $\mathcal{L}$ is the Langevin function $\mathcal{L}(x) = \coth(x) - 1/x$. The force diverges strongly as the extension approaches the contour length. A more complete discussion of this can be found in the original work.[16]

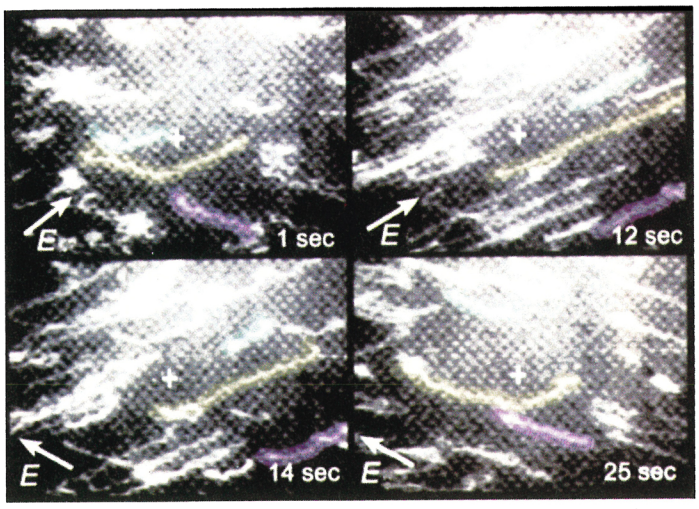


FIGURE 6. DNA MOLECULES ON A PEGBOARD, undergoing pulsed field electrophoresis with a 107° angle between the fields. Three lengths of DNA are highlighted, measuring 135 kilobases (yellow), 90 kilobases (violet) and 60 kilobases (blue). Immediately following a field switch, each molecule sets off in the new field direction led by what was previously its back end. The shortest molecule advances farthest along the bisector of the fields (vertically) during a complete pulse cycle. The longest molecule, by contrast, backtracks along its own path and makes no progress. (From Duke *et al.* in ref. 11.)

Molecule?" they speculated that this might be brought about by a change in the molecular architecture, in which tilting of the bases increased the overall length of the backbone. Nearly half a century later, the question posed in their title has been answered with an emphatic "Yes."

Studying DNA in action

Great versatility is demanded of the DNA molecule, since it must play many roles during the processes of transcription, replication, chromosome condensation and so on. The ability to switch between different structures undoubtedly aids the molecule in fulfilling its various tasks. For example, because the base pairs are much more exposed in S-DNA than in the normal B-DNA form of the molecule, it is possible that the transition is biologically significant for accessing the information contained in the DNA code.

Transcription of the DNA code into RNA has been investigated in some detail by Steve Block and his coworkers,[8] who immobilized an RNA polymerase on a surface and watched as it reeled a single DNA molecule through itself, while hydrolyzing ATP for energy. A small polystyrene bead was attached to the end of the DNA and trapped in an optical tweezer. The DNA became more taut as it was tugged by the polymerase, pulling the bead away from the center of the trap, until eventually the enzyme stalled. In this way, it was established that RNA polymerase is able to work against forces as high as 14 pN. Further experiments are under way, and the micromanipulation of single DNA molecules in the presence of proteins shows great promise. As well as shedding light on DNA transcription and recombination, these studies will help to elucidate how chromosomes are wound and condensed.

Putting stretched DNA to work

Aside from the examination of DNA mechanics *in vitro*, micromanipulation experiments have also contributed to the understanding of the behavior of DNA in important technological applications. The separation, purification and analysis of DNA molecules are essential parts of modern genetics, and more efficient methods are constantly being sought. Such tasks inevitably require that the molecules be transported by flow or electric fields. Knowledge of the static elastic properties gained from studies of single molecules helps us to understand how DNA responds dynamically to different flow conditions and to determine how the molecules will be deformed. For example, if a polymer is held still by one end in a uniform hydrodynamic flow, the drag causes it to extend.[9] This situation is more complicated than when a force is applied locally: In a flow, the tension in the polymer chain is zero at the free end and increases as one proceeds toward the tethering point. Flow studies are relevant to understanding gel electrophoresis, the method by which DNA molecules are sorted according to size.

In DNA electrophoresis, an electric field causes the molecules to migrate through a molecular mesh. The mesh (or matrix or network) may be made from either a gel or a concentrated solution of hydrophilic polymers, and is designed to restrict the motion of the DNA, essentially confining it to a "tube" along which it must wriggle. It was Pierre de Gennes who developed the "reptation" model of polymer dynamics that describes this situation.[10] That a tube indeed exists has been dramatically demonstrated by Steve Chu and his collaborators,[11] as shown in figure 5.

Reptation is the key to the success of gel electrophoresis. In the presence of a small enough force, the DNA molecule follows a contorted path through the gel; in the random-mesh topology, the molecule's mobility is inversely proportional to its length. Unfortunately, this technique for molecular migration only works with weak electric fields (less than about 10 V/cm) and short molecules (up to only about 20 kilobases). This is because the electric force acting on a polymer longer than that is strong enough to stretch the molecule and pull it out of the tube. Then the gel fibers, from which the tubes are constructed, can catch the DNA and temporarily anchor it, forcing the molecule to extend as though it were tethered. Subsequently, the DNA slides free of the obstruction and retracts elastically. The general motion is a complicated cycle of extension and relaxation, preventing any length-dependent mobility.

DNA on a pegboard

One way to study this problem is to replace the gel with a more controlled environment.[12] To that end, we fabricated a miniature obstacle course, an array of cylindrical posts on a silicon surface, made at Cornell's National Nanofabrication Facility. When sealed and filled with aqueous solution, the array is an almost two-dimensional electrophoretic chamber. Fluorescent DNA molecules can be observed moving through the array, transiently adopting elongated V-shaped conformations when they get

hooked over a post. This is analagous to what happens with the anchoring obstructions discussed above. These dynamics will not separate molecules according to size; the trick is to periodically change the direction of the electric field.

Figure 6 shows DNA being size segregated in a regular array of obstacles, using a field that alternates between two directions separated by an obtuse angle. This method has been applied in gels with some success,[13] but the dynamics observed in the arrays is much cleaner. Such microfabricated devices may permit the separation of intact human chromosomes (which is impossible to accomplish by gel electrophoresis) and facilitate genetic mapping.

Sequencing single DNA molecules

Although great progress has been made in sequencing technology, the task of sequencing the human genome remains formidable. The cost and time involved are staggering. Sequencing the genome of the virus *Hemophilus influenzae* cost about 50 cents per base pair for the 1.8 million base pairs and took several years. At that rate, sequencing the human genome would cost about $1.5 billion, which is not much when spread over the 30 or so centuries that would be needed.

It is a challenge to find new ways to sequence DNA, an area in which little progress has been made for many years. Ideally, one would like to stretch out a single molecule and read the base pairs directly, like reading the information from a ticker tape. To do so, it will be necessary to first handle very large DNA molecules, about the size of chromosomes. That has proved very difficult to do without breaking them, until recently.

Ted Cox[14] has developed a technology by which DNA can be accurately aligned on long, fine (5 μm thick) gold lines fabricated by electron-beam lithography, and subsequently elongated by hydrodynamic flow. (See figure 7.) David Bensimon and his coworkers[6] have discovered a way to stretch out DNA molecules on a treated glass surface, using the action of a receding meniscus, as shown on the cover of this issue. If one end of a molecule is attached to the surface and the drop of water in which the DNA is dissolved is allowed to evaporate, the molecule gets pulled out of the liquid as the meniscus retreats. The force acting on the DNA in the meniscus is strong, so when the portion of the molecule outside the water drop sticks to the surface, it is perfectly aligned. Occasionally, both ends are tethered to the glass, and the molecule gets initially aligned in two parallel lines, as in figure 4b. This process of "molecular combing" is not yet fully understood, but one can foresee some important applications of the technique.

The beauty of the combing method is that it allows the accurate alignment of a single DNA molecule on a surface on which one would hope that a physical technique, such as atomic force microscopy or scanning tunneling microscopy, might be used to resolve the individual bases of the stretched molecules. (See the article by Carlos Bustamante and David Keller in PHYSICS TODAY, December 1995, page 32.) Identifying single base pairs within a single molecule will be extraordinarily difficult. Some type of local spectroscopy of the molecule may prove to be valuable. The first steps in this direction have been taken by Cox's group,[15] who used a scanning tunneling microscope to study the chemical potential spectroscopy of free bases crystallized on a graphic surface. One day, a related technology may make it possible to scan rapidly along a single stretched DNA molecule and read its unique message.

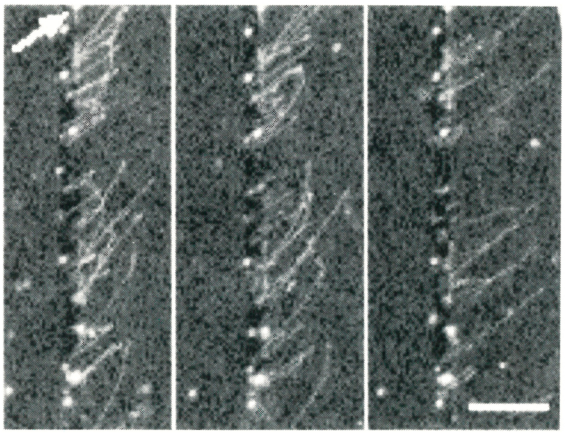


FIGURE 7. ALIGNING DNA MOLECULES. Fluorescent DNA molecules tethered to a microfabricated gold line (running vertically) and aligned in an electric field of 5 V/cm. The E field was switched 180° to the direction shown just prior to capturing the first frame. Frames are two minutes apart. The bar at the lower right is 15 μm long. (From ref. 14, and R. M. Zimmermann and E. C. Cox, unpublished.)

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