20. Bulk ooplasmic movements within living oocytes were assayed as follows: Adult females were transferred to a cover glass and covered with halocarbon oil, and egg chambers were removed and dissected as described (*19*). The cover glass was then transferred to the confocal microscope, and autofluorescent yolk granules were directly imaged with the BHS filter set provided with the Bio-Rad MRC 600 laser scanning confocal microscope. Single frame images were collected at 10-s intervals with the use of the confocal microscope with fluorescein filters. Temporal projections were created by summing 10 frames from a time-lapse sequence with the Project (maximum) utility of the COMOS software provided with the Bio-Rad 600 confocal microscope. Each projection represents 100 s of total elapsed time.
21. Thanks to T. Schüpbach for supplying *capu* and *spir* stocks and to P. Gergen, N. Dean, C. Polk, and the anonymous reviewers for helpful comments on the manuscript. Special thanks to L. Manseau for numerous lively discussions of these results.

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Alignment and Sensitive Detection of DNA by a Moving Interface

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In a process called "molecular combing," DNA molecules attached at one end to a solid surface were extended and aligned by a receding air-water interface and left to dry on the surface. Molecular combing was observed to extend the length of the bacteriophage λ DNA molecule to 21.5 ± 0.5 micrometers (unextended length, 16.2 micrometers). With the combing process, it was possible to (i) extend a chromosomal *Escherichia coli* DNA fragment (10^6 base pairs) and (ii) detect a minute quantity of DNA (10^3 molecules). These results open the way for a faster physical mapping of the genome and for the detection of small quantities of target DNA from a population of molecules.

Extension and manipulation of individual biopolymers is generally performed by first anchoring one end of the molecule at a solid matrix; stretching may then be achieved by viscous drag (*1*, *2*), electrophoresis (*3*), or optical forces (*4*). The method proposed here, which we call "molecular combing," extends a DNA molecule with a receding interface and fixes the molecule in this state on the dry substrate. This physical process leads to a complete, controlled, and reproducible alignment of all DNA fragments, thus allowing accurate position determinations along the molecule.

To anchor DNA to a glass surface, we first grafted a monolayer of silane molecules onto a glass cover slip by methods of "molecular self-assembly" (*5*), exposing a vinyl end group ($-CH=CH_2$) (*6*). These surfaces have the following characteristics: (i) a high binding specificity only for the ends of a DNA molecule—presumably because of the presence of a free protonated phosphate at the 5′ end—with a strong pH dependence, suggesting that the reaction between the molecules and the surface could be a case of electrophilic addition of weak acids to alkenes; (ii) the capability to bind proteins either directly or after oxidation to carboxylic acid groups; and (iii) a strong signal-to-noise ratio resulting from the negligible background fluorescence of glass. However, as usual with silanization procedures (*7*), the quality of the surface treatment is variable. As a result, the percentages of anchored DNA molecules and of their extension vary from batch to batch.

A drop of DNA solution (typically 5 μl) was deposited on a silanated cover slip. An untreated cover slip was then floated on top, forcing the drop to spread to a final thickness of ~20 μm. With video-enhanced fluorescence microscopy, molecules were observed (*8*) not only to be attached at one or both ends (*9*), as deduced from their extension by a flow or by electrophoresis, but also to fluctuate freely in solution (Fig. 1A), thereby indicating the absence of adhering nonspecific interactions between the surface and the molecule.

During evaporation of the DNA solution, the receding air-water interface left the bound molecules fully extended behind and deposited on the dried surface, whereas unbound molecules were swept by the moving interface. The temporal extension of a single fragment of *Escherichia coli* DNA molecule by a receding interface is shown in Fig. 1, B through D, and can lead to the alignment of a molecule 420 μm long (*10*), such as the one shown in Fig. 1E. "Molecular combing" seems to be an irreversible process: upon rehydration, combed molecules remain bound to the surface.

It turns out that the force the interface exerts on the DNA is strong enough to extend it but too weak to break the bond between the molecule and the surface. An estimate of the surface tension force on a rod of diameter D perpendicular to the surface of the interface is $F = \gamma\pi D$, where the surface tension $\gamma = 7 \times 10^{-2}$ N/m for the air-water interface. Because $D = 2.2$ nm for the DNA diameter, $F \approx 4 \times 10^{-10}$ N. This force is two orders of magnitude greater than the entropic forces keeping the DNA molecule in a random coil configuration (*2*) and is thus enough to fully extend the molecule, but it is apparently smaller than the force required to break a covalent bond (on the order of 10^{-9} N) (*11*). Details of the physics of the combing process will be presented elsewhere (*12*).

In contrast to viscous drag and electro-

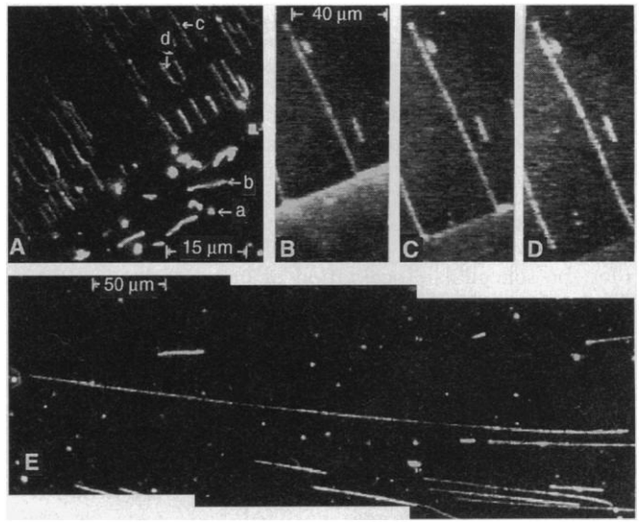

Fig. 1. Extension of DNA by a receding interface. (**A**) Video image showing λ DNA in solution (lower right part of image), bound at one (a) or both extremities (b). The interface extends across the image from the lower left to the upper right. The extended molecules left behind the interface are visible as straight segments (c), if bound at one end, or loops (d), if bound at both ends. (**B** through **D**) Time series showing the extension of a chromosomal *E. coli* DNA fragment by a receding interface (lower part of the image). The time between each pair of video images was 8.5 s. (**E**) Extension of an estimated 10^6–base pair fragment of chromosomal *E. coli*, reassembled from three video images (total length = 420 μm).

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phoresis, which act on the full length of the molecule, the action of the receding interface appears to be localized at the air-water interface and is thus independent of the length and conformation of the molecule. In comparison to results with other techniques, the combed molecules (i) are lying flat in a linearized state on a dry solid substrate, (ii) are observable by fluorescence even several months after combing, and (iii) are available for further manipulation. The local action of the interface is identical on all the molecules in solution: they are identically stretched. This is quantitatively shown by the distribution of lengths for a population of identical molecules that exhibits a well-defined peak at 21.5 ± 0.5 μm (see Fig. 2). The "background noise" in Fig. 2 is mainly due to a random fragmentation of the DNA molecules. That fragmentation process is, we believe, a result of the dye-induced fragilization of the molecule and the shear forces (*13*) during handling and combing. Partial stretching can be ruled out because short segments are not noticeably more fluorescent than long ones.

It appears that DNA combing on silanized glass surfaces provides a direct method to prepare samples for structural studies with scanning force microscopy (SFM) (*14*, *15*). From the averaged profile shown in Fig. 3, we deduce that the diameter of a stained combed molecule is 1.8 ± 0.8 nm (*16*), consistent with the expected value for the width of a double helix (2.4 nm unstained) (*17*).

The dry stretched molecules are uniquely distinguishable from all other uncorrelated fluorescence. By scanning the sample, one can easily determine the number of extended molecules. To test the sensitivity of the technique, we loaded decreasing amounts of DNA (10^5, 10^4, and 10^3 molecules) in a volume of 5 μl on silanized surfaces and then combed them (see Table 1). From the observed density of molecules per unit area, one can estimate that more than 70% of the molecules can be combed, depending on the quality of the surface treatment. Because the signal-to-noise ratio is independent of the number of molecules combed, the detection of a single molecule is limited only by the time required to scan the surface with the limited field of view of the objective. An attomole (10^{-18} mol = 6 × 10^5 molecules) of DNA is difficult to detect by methods not involving the polymerase chain reaction (PCR) (*18*) but represents a huge number of molecules easily detectable by the method presented here. Other less sensitive methods use radioactive (*19*) and nonradioactive probes (*20*), with sensitivities of the order of 10^{-18} mol.

Silanized surfaces have been coated with proteins to increase the binding specificity of DNA to the surface. By treating a silanized cover slip, first with protein A and then with monoclonal antibody to Digoxigenine (anti-Dig) (*21*), one obtains a surface that binds uniquely to end-labeled Dig-deoxyuridine-5′-triphosphate (dUTP) λ DNA (the rejection ratio between this Dig-labeled DNA and an unlabeled one is better than 10^5). Furthermore, the anchored DNA (with an estimated efficiency of better than 70%) could be combed; that is, the bond between Dig and anti-Dig is stronger than the tension exerted on the molecule by the receding interface (*12*).

Recently, the introduction of direct visualization hybridization (DIRVISH) maps, which is an improvement of fluorescence in situ hybridization (FISH) (*22*), has demonstrated that high-resolution multicolor maps can be attained by cell lysis and subsequent hybridization (*23*). With the use of molecular combing, a simple, controlled, and reproducible optical mapping on purified DNA molecules (for example, yeast artificial chromosomes) should be possible. After in vitro hybridization of DNA with probes stained with fluorescent antibodies or appropriate dyes and combing of the hybrid, it may be possible to measure distances with a resolution of 750 base pairs (250 nm) or better (*24*).

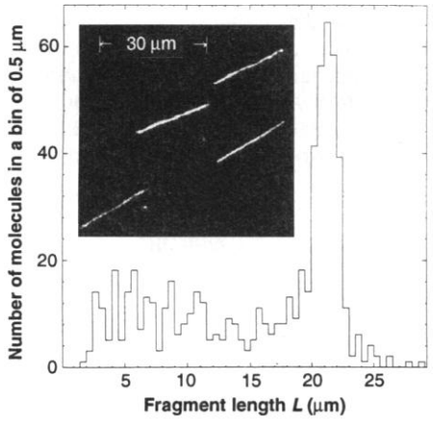

Fig. 2. Length distribution of extended λ DNA on silanized surfaces, compiled from 111 video images, containing 635 DNA segments. The peak at 21.5 μm (representing ≈50% of the molecules in the distribution) corresponds to a 33% increase over the unextended length of the molecule, 16.2 μm. (Inset) One of the 111 video images.

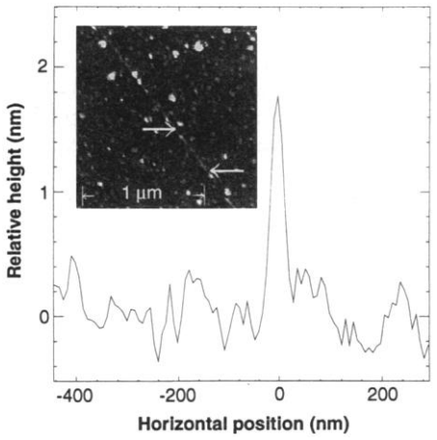

Fig. 3. Surface profile of a single combed DNA molecule scanned with a scanning force microscope. The noise in the image is due to the roughness of the cover slip glass (typically ±1.5 nm). In order to reduce that noise, the profile shown is an average over the profiles obtained from the 46 lines between the arrows in the atomic force microscope image shown in the inset.

Table 1. Detection sensitivity of three concentrations of stained λ DNA. The samples were manually and randomly scanned after combing. The deduced binding efficiency is >70%. This is a conservative estimate that takes into account a possible 33% increase in the number of DNA segments due to fragmentation by shear (see Fig. 2). A high binding efficiency is also consistent with the experimental observation that, while the interface recedes, the number of swept unbound molecules is always much smaller than the number of combed ones; f.o.v., field of view.

Dilution (no. of molecules)	f.o.v. (*n*)*	Total DNA segments	Expected mean DNA/f.o.v.	Sample mean DNA/f.o.v. ± SEM
10^5	77	397	5.2	5.16 ± 0.32
10^4	70	48	0.52	0.69 ± 0.09
10^3	122†	11	0.052	0.09 ± 0.03

*One f.o.v. = 1.3 × 10^{-4} cm^2. Total sample area = 2.5 cm^2. †This is a lower estimate because blank fields of view are difficult to count.

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6. Trichlorosilanes [$SiCl_3$–$(CH_2)_6$–CH = CH_2] were reacted with the SiOH groups on the cover glass, leaving the vinyl groups exposed. Cover glass surfaces were cleaned with an ultraviolet-ozone treatment (*7*), then transferred to a desiccator and flushed for 10

min with argon. Silane (300 μl) was added in a beaker near to the surfaces and left overnight to coat the cover glass. Immediately before use, the silanized surfaces were rinsed in deionized water and allowed to dry. We used λ DNA cI857 Sam7 (250 ng/μl) and bacterial chromosomal *E. coli* DNA (strain GN48 in 1% low-melting agarose plugs). The *E. coli* DNA concentration was determined by optical density to be 3.6 ng/μl. Both DNAs were stained in a 5:1 or 10:1 ratio [base pairs per dye (YOYO-1) molecule; Molecular Probes (Eugene, OR)]. Immediately before use, stained DNA was diluted in a 50 mM MES buffer (pH 5.5) to a final concentration of 0.2 pM. Typically, 10 μl of that solution was loaded onto a silanized cover slip. An unsilanized, but well-rinsed, cover slip then was floated gently on top and allowed to incubate at least 15 min at ambient temperature and humidity before examination on the microscope. The binding of DNA on silanized surfaces was dependent on the pH: the number of bound molecules in 50 mM MES buffer (pH = 5.5) is $\approx 10^4$ times that in 50 mM tris buffer (pH = 8.0). Video images were taken on an inverted fluorescence microscope equipped with a 60×, 1.4 numerical aperture objective, 0.9 to 2.25× video zoom, 100-W xenon source, optimized fluorescence filter set, and an intensified charge-coupled device camera. Video signals were averaged (typically 16 frames) and contrast-enhanced electronically by a commercial box and stored on tape. Images were digitized on a personal computer and analyzed with custom-written software.

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9. The probability of anchoring at both ends increases with incubation time.
10. This fragment (1 million base pairs) of DNA is covalently bound to the silanized surface at only one of its 5′ ends. All other methods of covalent (chemical) attachment of oligonucleotides to a surface that we know of lead to multiple parasitic bindings [see V. Lund, R. Schmid, D. Rickwood, E. Hornes, *Nucleic Acids Res.* **16**, 10861 (1988)].
11. This force is typically of the order of an electron volt per angstrom [see J. N. Israelachvili, *Intermolecular and Surface Forces* (Academic Press, San Diego, CA, 1985), pp. 24–28].
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13. For a molecule of length L and velocity v, the shear force is $F \approx \eta L v$, where η is the viscosity (10^{-2} poise for water). Taking a lower estimate for the rupture force to be $\approx 10^{-9}$ N, we find that shear velocities of a few hundred micrometers per second are enough to break an *E. coli* chromosomal DNA fragment.
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15. Stained λ DNA (7×10^6 molecules) was loaded onto a silanized surface and observed as described in Fig. 1. The molecules were then imaged with a scanning force microscope, in immersion oil with a standard pyramidal tip, V-shaped cantilever (100 μm long, 0.6 μm thick) with a spring constant k of 0.21 N/m. The piezoelectric tube has a 75-μm scanning range. The image shown is a part of a 4 μm by 4 μm scan (512 by 512 pixels). It was taken in topographic mode, at a 5-Hz line frequency.
16. From the full width at half maximum of the profile, one estimates the lateral resolution to be of the order of 30 nm, 10 times better resolved than with standard optical microscopy. Furthermore, we have verified that the DNA was fully linearized without knots by screening the length of a molecule by SFM.
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21. The right cohesive end of λ DNA was 3′ end-labeled with Dig-dUTP by terminal transferase and ligated to λ DNA. Silanized surfaces were treated with 60 μl of Protein A (1 mg/ml) in water for 2 hours at room temperature, rinsed three times in deionized water, and then incubated with 60 μl of anti-Dig solution (0.1 mg/ml) for 2 hours at room temperature. Surfaces were then rinsed three times in deionized water. Then 4 μl of YOYO-1–stained molecules (1.6 pM in 10 mM tris, pH = 8) were deposited on these prepared surfaces, covered with a rinsed unsilanized cover slip, and left to incubate in a humid atmosphere at room temperature for 1 hour, then left to dry.
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24. Furthermore, the possible detection of a single protein or antibody through coupling to a DNA "flag" and the subsequent combing of the bound DNA could be used to improve the sensitivity of protein detection, which is usually done by immunological assays (enzyme-linked immunosorbent assay) with a sensitivity of the order of 10^{-17} mol [see H. Labrousse *et al.*, *J. Immunol. Methods* **48**, 133 (1982)].
25. We thank J. P. Changeux, A. Ullmann, F. Rougeon, M. Hofnung, and M. Yaniv for scientific discussion and support. A.B. is a Human Frontier Science Postdoctoral Fellow. A.S. is a U.S. National Science Foundation Postdoctoral Fellow. Laboratoire de Physique Statistique and Laboratoire de Physique de la Matière Condensée are laboratories associated with the Centre National de la Recherche Scientifique and the Universités Paris 6 and Paris 7.

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Release of Adenosine by Activation of NMDA Receptors in the Hippocampus

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Adenosine is present in the mammalian brain in large amounts and has potent effects on neuronal activity, but its role in neural signaling is poorly understood. The glutamate receptor agonist *N*-methyl-D-aspartate (NMDA) caused a presynaptic depression of excitatory synaptic transmission in the CA1 region of guinea pig hippocampal slices. This depression was blocked by an adenosine A1 receptor antagonist, which suggests that activation of the NMDA subtype of glutamate receptor raises the concentration of extracellular adenosine, which acts on presynaptic inhibitory A1 receptors. Strong tetanic stimulation caused a heterosynaptic inhibition that was blocked by both NMDA and A1 receptor antagonists. Enkephalin, which selectively inhibits interneurons, antagonized the heterosynaptic inhibition. These findings suggest that synaptically released glutamate activates NMDA receptors, which in turn releases adenosine, at least in part from interneurons, that acts at a distance to inhibit presynaptically the release of glutamate from excitatory synapses. Thus, interneurons may mediate a widespread purinergic presynaptic inhibition.

Adenosine is a purine that is present in large amounts in the mammalian brain (*1*). When applied to brain slices, adenosine exerts a powerful presynaptic inhibition of excitatory synaptic transmission, which is mediated by A1 receptors (*2*). Furthermore, the concentration of extracellular adenosine is increased during hypoxia (*3*), synaptic stimulation (*4*), and the application of glutamate (*5*, *6*). The NMDA subtype of glutamate receptor is necessary for glutamate-evoked elevation of the concentration of adenosine, and this effect is Ca^{2+}-dependent (*6*). However, the source of this adenosine and the physiological mechanisms that control its concentration in the extracellular space are not known. We used here the hippocampal slice preparation in conjunction with electrophysiological techniques to address these issues.

We used standard techniques for preparing guinea pig hippocampal slices (*7*). Application of NMDA to the bath caused a depression in excitatory synaptic transmission, as described (*8*). Although the depression of field potentials is partly due to the direct depolarization of the postsynaptic cells, a number of observations indicate that this cannot fully account for the depression. First, the depression was of similar magnitude when the postsynaptic depolarization at the soma was prevented with whole-cell voltage clamping (Fig. 1). Second, the inhibition lasted longer than the inward current induced directly by NMDA (Fig. 1B; compare top and bottom graphs). Third, the inhibition was associated with a clear increase in paired-pulse facilitation (PPF) of excitatory transmission (Fig. 1, A and C) (227 ± 36% of the control, $n = 11$), a presynaptic phenomenon that is very sensitive to changes in transmitter release. Fourth, after the application of NMDA there was a marked, transient reduction of the frequency of spontaneous excitatory postsynaptic currents (EPSCs) ($n = 5$) (Fig. 1D). During this reduction in frequency, there was no significant change

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