# Scanning Force Microscopy of DNA Molecules Elongated by Convective Fluid Flow in an Evaporating Droplet

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ABSTRACT Scanning force microscopy (SFM) was used to image intact, nearly fully elongated lambda bacteriophage DNA molecules, fixed onto freshly cleaved mica surfaces. Molecular elongation and fixation were accomplished using a newly characterized fixation technique, termed "fluid fixation." Here convective fluid flows generated within an evaporating droplet of DNA solution efficiently elongate DNA molecules for fixation onto suitably charged surfaces. SFM images of a very large bacteriophage genome, G, showed the presence of double-stranded bubbles. We speculate that these structures may contain putative replication forks. Overall, the experiments presented here demonstrate the viability of using fluid fixation for the preparation of DNA molecules for SFM imaging. The combination of largely automatable optically based techniques with the high-resolution SFM imaging presented here will likely produce a high-throughput system for detailed physical mapping of genomic DNA or clones.

## INTRODUCTION

Scanning force microscopy (SFM), also called atomic force microscopy (AFM), has become an indispensable tool for the structural and biochemical investigation of biologically relevant macromolecules (Hansma and Hoh, 1994; Bustamante and Rivetti, 1996). The increasing use of SFM analysis in the biological sciences is not surprising, given its convenience and versatility in the gathering of molecular, and potentially even atomic-level, structural information of biological macromolecules. SFM has been most extensively used for the imaging of DNA molecules (Bustamante et al., 1992; Hansma et al., 1993, 1995, 1996a,b; Lyubchenko et al., 1993; Lyubchenko and Shlyyakhtenko, 1997; Henderson, 1992; Bezanilla et al., 1995; Mou et al., 1995; Yang and Shao, 1993) and DNA-protein complexes (Rees et al., 1993; Erie et al., 1994; Allison et al., 1996; Hansma, 1996), and has yielded new insights of biological interest. Examples include the SFM imaging of DNA bending in transcription complexes (Rees et al., 1993) and other DNA-protein complexes (Erie et al., 1994), RNA polymerase activity (Kasas et al., 1997), and the precise localization of *Eco*RI binding sites on plasmid DNAs (Allison et al., 1996). The rapid progress made in the development of new SFM techniques and applications has been facilitated in part by improved tip and sample preparation procedures. These developments will surely open new modes of biochemical and structural investigation.

Another area in which SFM may play an important role is the qualitative and quantitative analysis of biomolecular systems for the scanning and characterization of molecular entities. For example, many biological processes, such as replication and transcription, involve small molecular-scale intermediates with specific structural or conformational characteristics. Often the task is to map and identify such molecular-scale intermediates on a DNA chain that has a length many orders of magnitude larger. Traditionally, these structural or conformational features are biochemically isolated and studied by gel electrophoresis and electron microscopy. One important feature of SFM is that the sample preparation procedures are often similar to those used for optical microscopy, whereas its spatial resolution is comparable to that of electron microscopy. It can also be used to study biological systems in their natural environment, i.e., in water. Thus SFM has the potential of becoming a useful alternative to electron microscopy and gel electrophoresis. Although SFM has limited sample throughput, linking SFM to complementary imaging techniques offered by light microscopy may offer new approaches to molecular analysis. Optical imaging approaches to restriction endonuclease mapping of clones or even genomic DNAs such as optical mapping (Schwartz et al., 1993; Meng et al., 1995; Cai et al., 1995; Jing et al., submitted for publication) have obviated electrophoretic analysis in some applications. Optical mapping uses fluorescence microscopy to image individual DNA molecules after digestion with restriction endonucleases. DNA molecules are fluorochrome stained after digestion, so that cleavage sites appear as dark gaps created by the relaxation of newly formed molecular ends. A major emphasis of our laboratory in this direction is to integrate SFM techniques with optical mapping approaches to simultaneously image (using light and SFM techniques) biologically relevant structural features on concurrently acquired restriction maps. Such future studies may also be used to produce higher resolution restriction maps (Allison et al., 1996) or serve as the basis for new sequence acquisition approaches.

SFM is a surface-based imaging technique that requires the sample molecules to be mounted on a flat surface. Aggregation of molecules or entanglement of a single linear

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molecule will result in potentially observable molecular features being obscured. Such occurrences are hardly avoidable when studying physical properties of large DNA molecules. Without proper sample handling, these long macromolecules tangle, making the observation of structural features difficult (Hansma, 1996). Recently, DNA elongation has attracted much attention. Several physical methods have been employed to stretch DNA molecules; these include laser tweezers (Smith et al., 1996; Strick et al., 1996; Baumann et al., 1997), electric field (Volkmuth and Austin, 1992; Zimmerman and Cox, 1994), as well as fluid flow (Meng et al., 1995; Cai et al., 1995; Jing et al., manuscript submitted for publication; Bensimon et al., 1994, 1995; Hu et al., 1996; Perkins et al., 1995). Various fluid flow-induced DNA elongation and fixation techniques have been demonstrated (Meng et al., 1995; Cai et al., 1995; Jing et al., manuscript submitted for publication; Bensimon et al., 1994, 1995; Hu et al., 1996). In one method, the solvent flow for the elongation of DNA molecules was created by tilting the substrate (Cai et al., 1995). In another method, surface-grafted DNA molecules were stretched by the hydrodynamic force of a receding meniscus (Bensimon et al., 1994, 1995; Hu et al., 1996). Fluid flow within an evaporating droplet has also been shown to be capable of elongating and aligning DNA molecules (Jing et al., manuscript submitted for publication). In this method, termed *fluid fixation*, we determined that the elongation was due to the velocity gradient of the convective fluid flow caused by the evaporation of the droplet, coupled with interactions of the translating coil with the adsorbent surface. Fluid fixation has yielded well-elongated and aligned DNA molecules, which have been used in the construction of high-resolution restriction maps by optical mapping (Jing et al., manuscript submitted for publication). The simplicity of these procedures should make them valuable tools for the manipulation of DNA molecule conformation in other applications.

The chemical and physical characteristics of surfaces also play a significant role in DNA elongation and fixation. In the above-mentioned studies, substrate surfaces have been derivatized to provide DNA anchoring sites, consisting of, for example, positively charged aminosilane compounds covalently attached to glass surfaces. Surface-anchoring sites are required to attach elongated DNA molecules to surfaces in an aqueous environment. To achieve optimal elongation, however, a microscopically controlled surface charge distribution is necessary. For SFM applications, it is also necessary that the derivatized surfaces have atomic flatness. These surface issues have not yet been completely solved. Fortunately, much analytical SFM imaging can be performed under atmospheric conditions. For imaging in air, mica is one of the most convenient substrates. Atomically flat surfaces over a large area can be easily obtained by peeling a top layer off a mica substrate. Although underivatized mica surfaces do not bind DNA molecules with sufficient adhesion for aqueous imaging in most commonly used biological buffers, introducing certain divalent transition metal cations into the solution may lead to strong binding of DNA molecules to mica surfaces (Bezanilla et al., 1994; Hansma et al., 1996a; Kasas et al., 1997). However, these transition metal cations may not be compatible with biological processes, and complicated procedures may be required to allow biological activities (Kasas et al., 1997). Divalent metal ions may also cause significant perturbations to DNA structures, especially large DNAs (Duguid et al., 1993, 1995). On the other hand, mica does bind DNA molecules tightly in air when biological compatible cations exist. It is of interest, therefore, to investigate if such surface cations also support fluid fixation of DNA molecules to mica.

Here we report on the SFM imaging of fluid fixed DNA molecules on freshly cleaved mica surfaces. Intact lambda DNA molecules have been elongated and imaged. Preliminary results from the SFM imaging of bacteriophage G DNA molecules are also presented.

## MATERIALS AND METHODS

### Lambda bacteriophage DNA preparation for SFM

Lambda bacteriophage DNA (New England BioLabs, Beverly, MA) was diluted to 0.05 $\mu$g/ml in TE buffer (10 mM Tris, 1 mM EDTA, pH 7.6) with 0.02% (v/v) Triton X-100 detergent (Boehringer Mannheim, Indianapolis, IN). This concentration was determined by optical microscopy to give a fairly large number of DNAs on the surface without causing aggregation. Droplets of solution were spotted on a freshly cleaved ruby mica (Ted Pella, Redding, CA) surface with a pipette tip. The size of the droplet was controlled to be ~1 mm in diameter. Because the velocity of the convective flow within the droplet increases radially, by adjusting the droplet size, we can optimize the stretching forces (Jing et al., manuscript submitted for publication). The droplets were air-dried on a heating block at 60°C. Samples were then mounted on the SFM sample holder for imaging. Lambda DNA molecules for SFM imaging were not stained.

### Lambda DNA preparation for fluorescence microscopy

For fluorescence microscopy, lambda DNA prestained with oxazole yellow homodimer (YOYO-1; Molecular Probes, Eugene, OR) (0.1 $\mu$M in high-purity water) was prepared on mica surfaces in a procedure similar to that described above. After the droplet dried, samples were stored in a desiccator overnight or longer and then heated on a heating block at ~60°C for 1–2 h. The samples were then washed with isopropanol several times. A glass coverslip and 20% $\beta$-mercaptoethanol solution was used to seal the mica surface with prestained DNA for fluorescence imaging on an optical microscope. The dehydration step before fluorescence imaging was necessary to prevent DNA from rapidly detaching from the mica surface. With this dehydration step, fluid elongated and fixed DNA molecules remained on the surface long enough to allow the acquisition of fluorescence images.

### G bacteriophage preparation for SFM

G bacteriophage (Carolina Biological Supply Co., Burlington, NC) was grown in the laboratory. G phage DNA was extracted by a procedure that was a modification of the procedure described by Fangman (1978). The G phage DNA integrity and purity were confirmed by pulsed field gel electrophoresis (PFGE) sizing (Schwartz and Cantor, 1984). For SFM imaging, G phage DNA of ~20 $\mu$g/ml was pipetted onto a freshly cleaved mica substrate. The concentration of the G DNA sample was adjusted in a way similar to that of lambda DNA samples. A glass coverslip was put on

the mica after the G phage DNA solution had been deposited. The free weight of the coverslip caused the sandwiched solution to spread. In cases where a stronger fluid flow was necessary, an appropriate weight can also be added to the coverslip. After the spreading of the solution, the coverslip was removed immediately and the sample was allowed to dry in air. With this procedure, we prepared well-elongated and aligned DNA molecules routinely. After drying, the samples were mounted on the SFM for imaging. The G phage DNA molecules were not stained.

### Scanning force microscopy

The scanning force microscope used in the experiments was a BioScope (Digital Instruments, Santa Barbara, CA) mounted on an inverted optical microscope (Axiovert 100; Carl Zeiss, Thornwood, NY). The combined system was mounted on an air suspension table via silicone patches. No additional acoustic or air flow insulation was provided. High-quality DNA images could be obtained routinely under these conditions in both contact mode and tapping mode. All images presented in this paper were collected in tapping mode in air with standard (unsharpened) silicon tips from Digital Instruments.

### Fluorescence microscopy

Fluorescence images were taken by an inverted optical microscope equipped for epifluorescence with a 100× Zeiss plan-neufluar oil immersion objective (Axiovert 135; Carl Zeiss). The fluorescence images were collected by a cooled CCD camera (1316 × 1032 pixels; Princeton Instruments, Trenton, NJ). The lengths of elongated DNA were measured by using IPLab Spectrum (Signal Analytics; Vienna, VA).

## RESULTS AND DISCUSSION

The 48.5-kb lambda DNA molecule has a B-form contour length of ~16.5 $\mu$m (using an upper limit of 0.34 nm/bp). Without proper elongation procedures, such molecules appear as tangled masses on surfaces when imaged by both SFM and light microscopy. The difficulty in preparing lambda DNA samples has also hindered attempts at SFM physical mapping of lambda DNA (Allison et al., 1996). Previously, entire lambda DNA molecules were only captured by SFM in unelongated form (Lyubchenko et al., 1993; Hansma et al., 1996b). In this work, with the fluid fixation technique being applied to elongate these long DNA molecules in the sample preparation step, entire lambda DNA molecules could be reliably and routinely imaged. In Fig. 1 we present typical SFM images of fully extended lambda DNA molecules. Fig. 1 *a* shows a lambda DNA that was uniformly elongated to ~15.7 $\mu$m—very close to its full contour length. Fig. 1, *b–d*, shows more images of fluid-fixed lambda DNA molecules (see Materials and Methods). The alignment of these lambda DNA molecules along the fluid flow direction is obvious. The majority of the fluid-fixed lambda DNA molecules imaged by SFM have lengths between 13 and 14 $\mu$m, which is ~80–85% of the calculated polymer contour length. Currently we cannot generate a statistically reliable histogram of DNA length distribution by SFM, because of a combination of SFM's low throughput and the very low DNA density used in the experiments. This will be best done when fluorescence microscopy is interfaced with SFM, to allow more efficient zooming to molecules. Lambda DNA molecules elongated and fixed on freshly cleaved mica by the same fluid fixation procedure were also imaged by fluorescence microscopy. Fig. 2 shows a representative fluorescence micrograph. The degree of elongation is consistent with the SFM results. This degree of elongation is also consistent with previous results (Jing et al., manuscript submitted for publication) on fluid elongated and fixed adenovirus 2 DNA, where an average extension to ~80% of the DNA contour length was observed by optical microscopy. It should be noted here that this degree of elongation is not an intrinsic property of the fluid fixation technique, but is our own choice. By adjusting the fluid flow and surface characteristics, the degree of DNA elongation can be varied. For example, in optical mapping applications, the degree of DNA elongation was chosen to preserve biochemical activities of the elongated DNA molecules.

The apparent height of these fluid-fixed lambda DNAs was ~1 nm. As examples, the molecule in Fig. 1 *a* had an apparent height of 0.98 ± 0.20 nm, and the molecule in Fig. 1 *c* had an apparent height of 1.17 ± 0.28 nm. These numbers were smaller than both the 2-nm B-form DNA diameter and the previous SFM measurements of ~1.8 nm on segments of DNA elongated by molecular combing (Bensimon et al., 1994). The deviation may be attributed to the salt deposition, which increased the background significantly. The widths at the base for the DNA molecules in Fig. 1, *a* and *c*, are 147.6 ± 49.1 nm and 82.8 ± 15.6 nm, respectively. These numbers are much larger than the DNA diameter of 2 nm. Although tip radii may play a role in the increased width measurements, the major reason for the exaggeration of DNA width here came from the large scan sizes we used.

The imaging of these fluid-fixed DNA molecules was very stable. Repetitive scanning, including zooming and moving the scan origin, generated consistent images. The same surface area could be scanned for several hours with no discernible structural perturbations to the DNA molecules being observed. This indicates that elongated DNA molecules are tightly bound to the mica surface. No damage to DNA molecules due to scanning operations was observed, even at much higher magnification than the images reported here. This may be an advantage of tapping mode over contact mode for biological imaging. Surprisingly, image quality is quite good, even in the absence of sophisticated noise reduction measures, such as isolation from acoustic noise. From the images in Fig. 1 it can be seen that the background noise level was indeed high. In addition, the adverse effects of surface roughness and the scanner's upper line limit to only 512 lines were more serious in such large scans. Nonetheless, we were able to reproducibly image fluid-fixed DNA molecules. Other factors also affect the quality of the images. For example, in Fig. 1 *a*, the blur around one end of the DNA molecule was due to the tip picking up contaminant during the scan. The DNA image in Fig. 1 *b* shows a rougher profile. This sample had been dried in a desiccator for more than 24 h before imaging. These

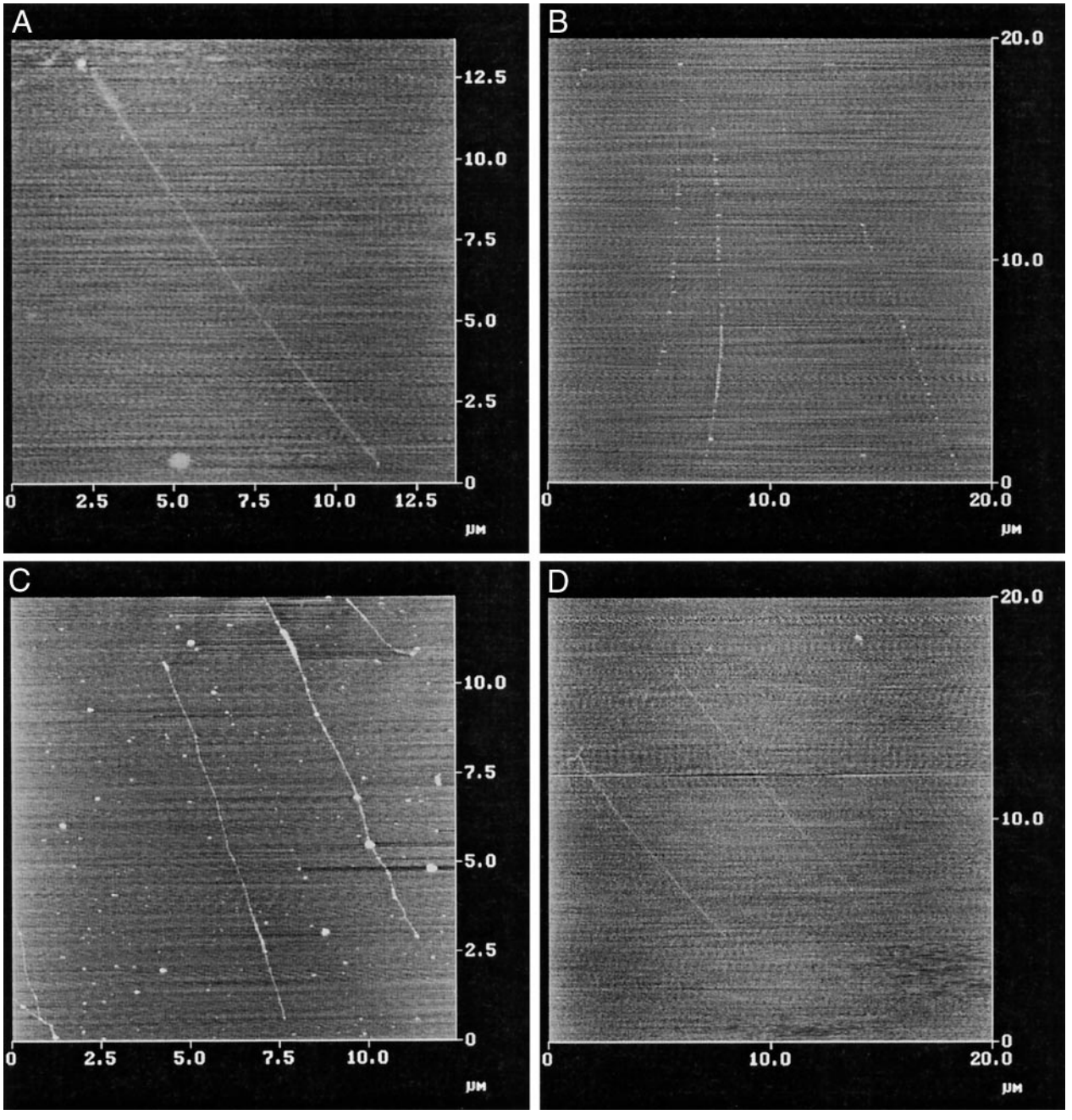


FIGURE 1 (*a*) SFM image of a fluid-fixed lambda DNA on freshly cleaved mica surface. The length of the molecules is 15.7 $\mu$m. (*b*) SFM image of two fluid-fixed lambda DNA molecules. Their lengths are 14.0 $\mu$m (*left*) and 15.2 $\mu$m (*right*), respectively. The sample in this image was dried in a desiccator for 24 h before imaging. (*c*) SFM image of a less than fully extended lambda DNA. The length of the molecule is 10.6 $\mu$m. Nonuniform elongation in this molecule is clearly shown by its brighter, less stretched end (*top end*) and an internal section. (*d*) SFM image of another two lambda DNA molecules. Their lengths are 12.6 $\mu$m (*top*) and 10.5 $\mu$m (*bottom*), respectively. Note the that shorter molecule has an unstretched end.

DNA molecules may have been more tightly bound to the surface and, therefore, followed the rough surface terrain more closely. These problems are not fundamental and will be solved when more favorable imaging conditions are provided. Because an atomically flat surface can be obtained by simply lifting a layer off a mica sheet, for many SFM imaging applications, freshly cleaved mica remains the most convenient choice of substrate. The simple method for preparing well-elongated and aligned DNA molecules on mica will add new capability to SFM imaging of DNA molecules and other biomacromolecular samples.

In addition to length information, SFM images of DNA also provide high-resolution width and height information. Using such information, it is possible to examine the degree of uniformity in DNA elongation (Thundat et al., 1994). Fig. 1 *c* shows an example of nonuniform elongation along a single DNA molecule, with an apparent length of 10.6 $\mu$m. The SFM image shows nonuniform elongation, evi-

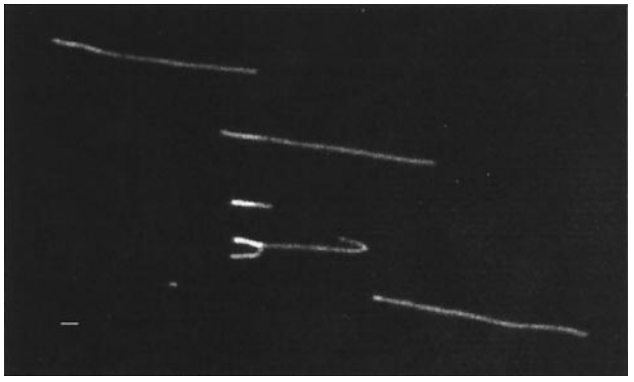

FIGURE 2 Fluorescence micrograph image of fluid-fixed lambda DNA on freshly cleaved mica surface. The molecular lengths are 12.8 $\mu$m, 13.5 $\mu$m, 13.6 $\mu$m, respectively (*top to bottom*). An intact, but not fully elongated DNA can also be seen. The molecular length of this unstretched lambda DNA is 14.2 $\mu$m. The scale bar represents 1 $\mu$m.

denced by segments that are both wider and taller. These segments, one at the top end and the other in the lower interior (Fig. 1 *c*), may have a lower degree of elongation compared to other parts of the molecule. A similar observation can be made of the bottom molecule in Fig. 1 *d*, which shows incompletely elongated end. We do not know if this inhomogeneity in DNA length is a result of nonuniform elongation or of relaxation on the surface, because neither the surface charge distribution nor the fluid flow pattern is well characterized. Thundat and co-workers have also observed nonuniform stretching with sections of DNA stretched as much as 80% beyond their normal polymer contour length (Thundat et al., 1994). In their case, because a stretched section was connected at the two ends to tangled DNA masses, relaxation was less likely and the stretching should be due to the hydrodynamic drag forces. We have used much milder flow fields, as evidenced by the fact that we did not observe overstretched DNAs under conditions used in this work; nonetheless, nonuniform elongation has been observed, indicating that DNA stretching behavior is very sensitively determined by DNA/surface interactions and DNA/flow interactions. This issue will be most easily addressed when we have more precise knowledge and experimental control over these two aspects of fluid fixation techniques. SFM has been demonstrated to resolve helical turns (Hansma et al., 1995; Mou et al., 1995); thus we can expect it to be an excellent tool for fine mapping of segmental density across individual fixed molecules.

These experiments have demonstrated that fluid fixation of large DNA molecules on freshly cleaved mica is possible. In earlier experiments, derivatized glass or mica surfaces were used to provide DNA anchoring sites (Meng et al., 1995; Cai et al., 1995; Jing et al., manuscript submitted for publication; Bensimon et al., 1994, 1995; Hu et al., 1996). In those experiments, it was shown that elongation was a result of an interplay between DNA anchoring, mediated by the electrostatic interactions between the negatively charged DNA molecules and a positively charged surface, e.g., aminosilane derivatized glass (Cai et al., 1995; Jing et al., manuscript submitted for publication) or mica surface (Hu et al., 1996), and the hydrodynamic drag on DNA molecules caused by the fluid flow. Similar mechanisms could be responsible for the elongation on freshly cleaved mica surfaces. For the muscovite type of mica used in this work, charge balance is maintained by cations, predominantly potassium ion, in the interlayer sites (Gaines, 1957). In the solid phase, these cations serve as bridges to help bind the two negatively charged mica layers together. We speculate that these cation sites may play the role of anchoring sites for DNA adhesion by serving as the bridges between the negatively charged mica surface and the negatively charged DNA molecules. The force between DNA molecules and these cations sites, albeit much weaker than the force between DNA molecules and, e.g., aminosilane, may be enough for fluid fixation. In the case of a constant fluid flow, it is likely that the existence of a derivatized surface that provides strong DNA adhesion points (anchoring sites) would be necessary for the elongation of DNA molecules. However, if the flow field has a velocity gradient, as in the case of a convective flow field in a drying droplet, DNA molecules are partially elongated before being immobilized on a surface. Video microscopy of the elongation process of YOYO-1-stained DNA molecules during droplet drying has shown evidence of this mechanism of DNA elongation (Jing et al., manuscript submitted for publication). It is also possible that different mechanisms contribute to the elongation of DNA molecules, and under different conditions the dominant mechanism could be different. Hansma and co-workers have demonstrated that certain divalent transition metal ions, such as $Ni^{2+}$ and $Zn^{2+}$, may enhance DNA binding to mica surfaces (Bezanilla et al., 1994; Hansma et al., 1996a; Kasas et al., 1997). These cations, on the other hand, are also known to perturb the structure of DNA molecules significantly and to provoke DNA condensation (Duguid et al., 1993, 1995; Bloomfield, 1996; Baumann et al., 1997). It will be of interest to see if these cations will support the elongation and fixation of DNA molecules on mica for SFM imaging in aqueous solutions.

To demonstrate the potential of combining fluid fixation with SFM, we next present preliminary results of SFM imaging of bacteriophage G DNA. G phage is a very large bacteriophage, containing a linear 670-kb DNA molecule (Sewer et al., 1995; Hutson et al., 1995; Donelli et al., 1975). Direct deposition of such a large coil of DNA on a surface without elongation would yield samples that are unsuitable for SFM studies aimed at resolving internal structural features. Fig. 3 *a* shows an image of fluid-fixed G phage DNA molecules. Sections of three molecules were captured in this image. Again, all molecules are apparently well elongated and aligned along the fluid flow direction. The apparent height of 1.84 $\pm$ 0.42 nm revealed that they are single helices of double-stranded DNA molecules. The width at the base was 47.6 $\pm$ 7.6 nm. In Fig. 3 *a* we can also see "bubbles" in the interior of these elongated DNA molecules. The extended form of DNA used simplifies the

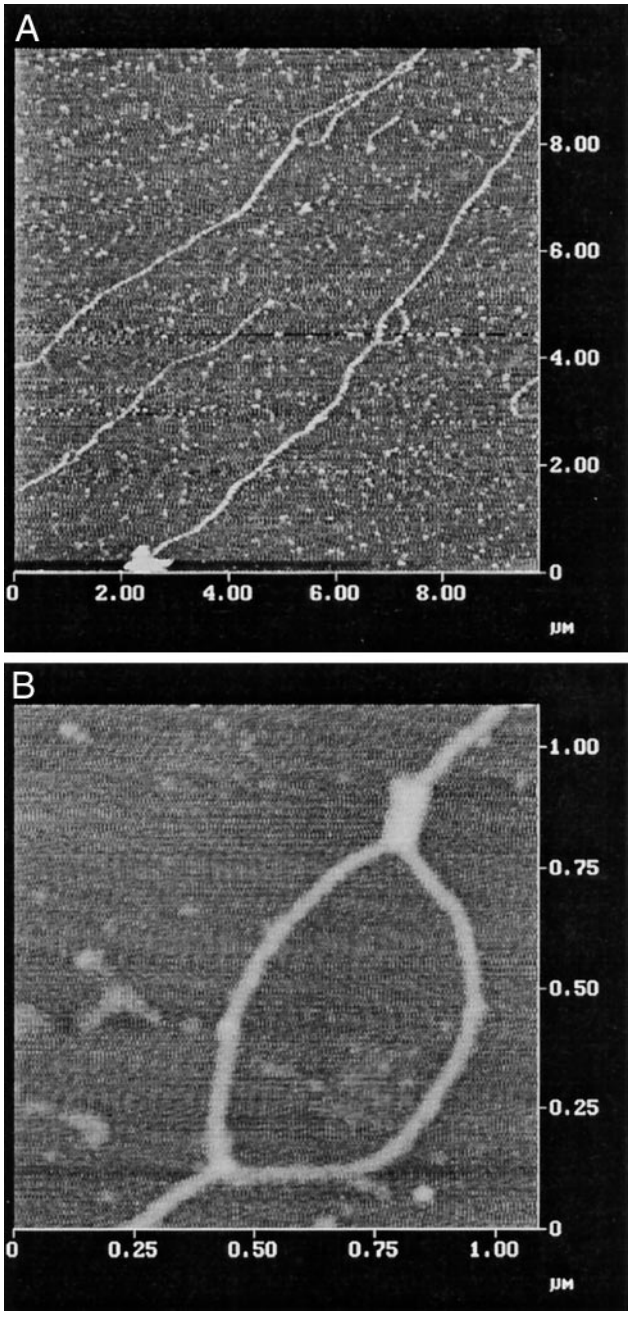


FIGURE 3 (*a*) SFM image of fluid flow elongated G phage DNA molecules on mica surface. (*b*) A higher magnification scan of a bubble in *a*.

unambiguous identification of these features. We tentatively identified them as replication bubbles, because the two arms of each of these "bubbles" have the same height and width (1.90 ± 0.39 nm and 46.0 ± 9.0 nm, respectively) as a single double-stranded DNA. Further evidence supporting the two bubble arms as double-stranded comes from the apparent rigidity and smoothness of these bubble arms. Single-stranded DNA molecules are usually much less rigid and tend to form branches of base-pairing regions (see for example, Hansma, 1996). Based on their heights and widths, we can also argue against that these bubbles are loops in a multistrand DNA bundle. If they are loops in an otherwise multistrand bundle, we should expect to observe significant differences in the height and width between the loop arms and the main strand. Considering that our samples have been carefully prepared to avoid DNA aggregation (see Material and Methods), it becomes even less possible that the images were DNA bundles. The exact replication mechanism of G phage is not known. The initial stage of replication for most linear DNAs, however, should involve the formation of such replication bubbles (Kornberg and Baker, 1992; Godson, private communication). A conclusive assignment of these as replication bubbles would require further experiments, including better experimental controls to enrich replication intermediates during the DNA preparation (Wolfson et al., 1971; Dressler et al., 1971). These experiments, in combination with the physical and genetic mapping of this complex genome (Dore et al., 1977), will shed light on the replication mechanism of this phage. Here our major goal is to demonstrate the utility of SFM as a qualitative analytical tool when combined with molecular manipulation in sample preparation for the observation of small molecular structures. The putative replication bubbles shown here have dimensions near the resolution limit of optical microscopy, but can clearly be observed by SFM without the use of sharpened tips and more sophisticated noise reduction measures. Fig. 3 *b* shows a higher magnification scan of the bubble in the bottom of the image in Fig. 3 *a*. A twist of the upper arm can be seen. This is possibly because when this DNA landed on the surface, the bubble plane was not parallel to the surface.

One of the goals of this study was to explore the possibility of preparing surface-mounted DNA samples for reproducible SFM imaging using only the common sample preparation protocols in optical mapping. The results are quite satisfactory. These encouraging results open the way for the incorporation of SFM techniques into our optical mapping procedures. Optical mapping has been demonstrated to be a powerful approach for the construction of high-resolution restriction maps (Schwartz et al., 1993; Meng et al., 1995; Cai et al., 1995; Jing et al., manuscript submitted for publication). One major advantage of optical mapping is that the spatial order of restriction fragments is maintained after enzyme digestion. This offers an enormous opportunity for the development of a combined SFM and optical mapping-based DNA analysis approach to quickly locate and study biologically important sites on a DNA. Examples include locating replication origins or DNA heteroduplex mapping of mutations such as substitutions and deletions (Westmoreland et al., 1969; Kim et al., 1972). In the latter application, the mismatch sequence sites or deletion sites should show up as single-stranded bubbles along a double-stranded heteroduplex DNA and can be resolved by SFM. It should be noted that because SFM has the potential of achieving atomic resolution, a single base-pair bubble should be observable. The distance between such a site and restriction sites can then be used to determine the location of sites of interest on a physical map. Alternatively,

if the location of a site of interest is known, optical mapping will allow us to zoom to the exact restriction fragment directly. This is particularly useful for studying small features in a long section of DNA.

## CONCLUSIONS

Large DNA molecules have been elongated and aligned on freshly cleaved mica surfaces by the fluid flow developed within a drying droplet of DNA solution. SFM was used to image these fluid flow elongated DNA molecules. Entire lambda DNA molecules elongated to close to their contour length were imaged. Evidence of nonuniform elongation was also observed and discussed. Elongated G phage DNAs have also been imaged with replication bubbles in their interiors. These experiments demonstrated the utility of combining fluid fixation and SFM in the imaging of large polymer chains such as DNA. The experiments also point the way to the integration of SFM and optical mapping for the development of new approaches to DNA analysis.

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## REFERENCES

Allison, D. P., P. S. Kerper, M. J. Doktycz, J. A. Spain, P. Modrich, D. W. Larimer, T. Thundat, and R. J. Warmack. 1996. Direct atomic force microscope imaging of *Eco*RI endonuclease site specifically bound to plasmid DNA molecules. *Proc. Natl. Acad. Sci. USA.* 93:8826–8829.

Baumann, C. G., S. B. Smith, V. A. Bloomfield, and C. Bustamante. 1997. Ionic effects on the elasticity of single DNA molecules. *Proc. Natl. Acad. Sci. USA.* 94:6185–6190.

Bensimon, A., A. Simon, A. Chiffaudel, V. Croquette, F. Heslot, and D. Bensimon. 1994. Aligment and sensitive detection of DNA by a moving interface. *Science.* 265:2096–2098.

Bensimon, D., A. Simon, V. Croquette, and A. Bensimon. 1995. Stretching DNA with a receding meniscus: experiments and models. *Phys. Rev. Lett.* 74:4754–4757.

Bezanilla, M., B. Drake, E. Nudler, M. Kashlev, P. K. Hansma, and H. G. Hansma. 1994. Motion and enzymatic degradation of DNA in the atomic force microscope. *Biophys. J.* 67:2554–2559.

Bezanilla, M., S. Manne, D. E. Laney, Y. L. Lyubchenko, and H. G. Hansma. 1995. Adsorption of DNA to mica, silylated mica, and minerals: characterization by atomic force microscopy. *Langmuir.* 11: 655–659.

Bloomfield, V. A. 1996. DNA condensation. *Curr. Opin. Struct. Biol.* 6:334–341.

Bustamante, C., and C. Rivetti. 1996. Visualizing protein-nucleic acid interactions on a large scale with the scanning force microscope. *Annu. Rev. Biophys. Biomol. Struct.* 25:395–429.

Bustamante, C., J. Vesenka, C. L. Tang, W. Rees, M. Guthold, and R. Keller. 1992. Circular DNA molecules imaged in air by scanning force microscopy. *Biochemistry.* 31:22–26.

Cai, W., H. Aburatani, V. P. Stanton, Jr., D. E. Housman, Y. K. Wang, and D. C. Schwartz. 1995. Ordered restriction endonuclease maps of yeast artificial chromosomes created by optical mapping on surfaces. *Proc. Natl. Acad. Sci. USA.* 92:5164–5168.

Donelli, G., E. Dore, C. Frontali, and M. E. Grandolfo. 1975. Structure and physico-chemical properties of bacteriophage G III. A homogeneous DNA of molecular weight $5 \times 10^6$. *J. Mol. Biol.* 94:555–565.

Dore, E., C. Frontali, and M. Grignoli. 1977. The molecular complexity of G DNA. *Virology.* 79:442–445.

Dressler, D., J. Wolfson, and M. Magazin. 1971. Initiation and replication of DNA synthesis during replication of bacteriophage T7. *Proc. Natl. Acad. Sci. USA.* 69:998–1002.

Duguid, J. G., V. A. Bloomfield, J. M. Benevides, and G. J. Thomas, Jr. 1993. Raman spectroscopy of DNA-metal complexes. I. Interactions and conformational effects of the divalent cations: Mg, Ca, Sr, Ba, Mn, Co, Ni, Cu, Pd, and Cd. *Biophys. J.* 65:1916–1928.

Duguid, J. G., V. A. Bloomfield, J. M. Benevides, and G. J. Thomas, Jr. 1995. Raman spectroscopy of DNA-metal complexes. II. The thermal denaturation of DNA in the presence of $Sr^{2+}$, $Ba^{2+}$, $Mg^{2+}$, $Ca^{2+}$, $Mn^{2+}$, $Co^{2+}$, $Ni^{2+}$, and $Cd^{2+}$. *Biophys. J.* 69:2623–2641.

Erie, D. A., G. Yang, H. C. Schultz, and C. Bustamante. 1994. DNA bending by Cro protein in specific and nonspecific complexes: implications for protein site recognition and specificity. *Science.* 266: 1562–1566.

Fangman, W. L. 1978. Separation of very large DNA molecules by gel electrophoresis. *Nucleic Acids Res.* 5:653–666.

Gaines, Jr., G. L. 1957. The ion-exchange properties of muscovite mica. *J. Phys. Chem.* 61:1408–1413.

Hansma, H. G. 1996. Atomic force microscopy of biomolecules. *J. Vac. Sci. Technol.* B14:1390–1394.

Hansma, H. G., M. Bezanilla, F. Zenhausern, M. Adrian, and R. L. Sinsheimer. 1993. Atomic force microscopy of DNA in aqueous solutions. *Nucleic Acids Res.* 21:505–512.

Hansma, H. G., and J. Hoh. 1994. Biomolecular imaging with atomic force microscope. *Annu. Rev. Biophys. Biomol. Struct.* 23:115–39.

Hansma, H. G., and D. E. Lancey. 1996a. DNA binding to mica correlates with cationic radius: assay by atomic force microscopy. *Biophys. J.* 70:1933–39.

Hansma, H. G., D. E. Laney, M. Bezanilla, R. L. Sinsheimer, and P. K. Hansma. 1995. Applications for atomic force microscopy of DNA. *Biophys. J.* 68:1672–1677.

Hansma, H. G., I. Revenko, K. Kim, and D. E. Laney. 1996b. Atomic force microscopy of long and short double-stranded, single-stranded and triple-stranded nucleic acids. *Nucleic Acids Res.* 24:713–720.

Henderson, E. 1992. Imaging and nanodissection of individual supercoiled plasmids by atomic force microscopy. *Nucleic Acids Res.* 20:445–447.

Hu, J., M. Wang, H.-U. G. Weier, P. Frantz, W. Kolbe, D. F. Ogletree, and M. Salmeron. 1996. Imaging of single extended DNA molecules on flat (aminopropyl)triethoxysilane-mica by atomic force microscopy. *Langmuir.* 12:1697–1700.

Hutson, M. S., G. Holzwarth, T. Duke, and J.-L. Viovy. 1995. Two-dimensional motion of DNA bands during 120° pulsed-field gel electrophresis. I. Effect of molecular weight. *Biopolymers.* 35:297–306.

Kasas, S., N. H. Thomason, B. L. Smith, H. G. Hansma, X. Zhu, M. Guthold, C. Bustamante, E. T. Kool, M. Kashlev, and P. K. Hansma. 1997. *E. coli* RNA polymerase activity observed using atomic force microscopy. *Biochemistry.* 36:461–468.

Kim, J.-S., P. A. Sharp, and N. Davidson. 1972. Electron microscope studies of heteroduplex DNA from a deletion mutant of bacteriophage $\phi$X-174. *Proc. Natl. Acad. Sci. USA.* 69:1948–1952.

Kornberg, A., and T. A. Baker. 1992. DNA Replication, 2nd Ed. W. H. Freeman and Co., New York.

Lyubchenko, Y. L., and L. S. Shlyyakhtenko. 1997. Visualization of supercoiled DNA with atomic force microscopy in situ. *Proc. Natl. Acad. Sci. USA.* 94:496–501.

Lyubchenko, Y., L. Shlyakhtenko, R. Harrington, P. Oden, and S. Lindsay. 1993. Atomic force microscopy of long DNA: imaging in air and under water. *Proc. Natl. Acad. Sci. USA.* 90:2137–2140.

Meng, X., K. Benson, K. Chada, E. J. Huff, and D. C. Schwartz. 1995. Optical mapping of lambda bacteriophage clones using restriction endonucleases. *Nature Genet.* 9:432–438.

Mou, J., D. M. Czajkowsky, Y. Zhang, and Z. Shao. 1995. High-resolution atomic-force microscopy of DNA: the pitch of the double helix. *FEBS Lett.* 371:279–282.

Perkins, T. T., D. E. Smith, R. G. Larson, and S. Chu. 1995. Stretching of a single tethered polymer in a uniform flow. *Science.* 268:83–87.

Rees, W. A., R. W. Keller, J. P. Vesenka, C. Yang, and C. Bustamante. 1993. Evidence of DNA bending in transcription complexes imaged by scanning force microscopy. *Science.* 260:1646–1649.

Schwartz, D. C., and C. R. Cantor. 1984. Separation of yeast chromosome-sized DNAs by pulsed field gradient gel electrophoresis. *Cell.* 37:67–75.

Schwartz, D. C., X. Li, L. I. Hernandez, S. P. Ramnarain, E. J. Huff, and Y.-K. Wang. 1993. Ordered restriction maps of *Saccharomyces cerevisiae* chromosomes constructed by optical mapping. *Science.* 262: 110–114.

Sewer, P., A. Estrada, and R. A. Harris. 1995. Video light microscopy of 670-kb DNA in a hanging drop: shape of the envelop of DNA. *Biophys. J.* 69:2649–2660.

Smith, S. B., Y. Cui, and C. Bustamante. 1996. Overstretching B-DNA: the elastic response of individual double-stranded and single-stranded DNA molecules. *Science.* 271:795–798.

Strick, T. R., J.-F. Allemand, D. Bensimon, A. Bensimon, and V. Croquette. 1996. The elasticity of a single supercoiled DNA molecules. *Science.* 271:1835–1837.

Thundat, T., D. P. Allison, and R. J. Warmack. 1994. Stretched DNA structures observed with atomic force microscopy. *Nucleic Acids Res.* 22:4224–4228.

Volkmuth, W. D., and R. H. Austin. 1992. DNA electrophoresis in microlithographic arrays. *Nature.* 358:600–602.

Westmoreland, B. C., W. Szybalski, and H. Ris. 1969. Mapping of deletions and substitutions in heteroduplex DNA molecules of bacteriophage lambda by electron microscopy. *Science.* 163:1343–1348.

Wolfson, J., D. Dressler, and M. Magazin. 1971. Bacteriophage T7 DNA replication: a linear replicating intermediate. *Proc. Natl. Acad. Sci. USA.* 69:499–504.

Wyman, C., E. Grotkopp, C. Bustamante, and H. C. M. Nelson. 1995. Determination of heat-shock transcription factor 2 stoichiometry at looped DNA complexes using scanning force microscopy. *EMBO J.* 14:117–123.

Yang, J., and Z. Shao. 1993. Effect of probe force on the resolution of atomic force microscopy of DNA. *Ultramicroscopy.* 50:157–170.

Zimmerman, R. M., and E. C. Cox. 1994. DNA stretching on functionalized gold surfaces. *Nucleic Acids Res.* 22:492–497.