

Scanning tunneling microscopy of deoxyribonucleic acid during replication

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Scanning tunneling microscopy was used to produce topographic images of uncoated and unlabeled deoxyribonucleic acid (DNA) in air, on a graphite substrate. The images show for the first time a DNA molecule that had been isolated while it was replicating.

Previous scanning tunneling microscopy (STM) experiments have produced several images of deoxyribonucleic acid (DNA) molecules, but never that of DNA in the act of replicating. We present such an image, obtained on an uncoated and unlabeled molecule extracted during replication from the cell. The results were highly reproducible, and generally consistent with previously reported cases of STM imaging of coated or uncoated DNA.¹⁻⁸ Our micrographs show the parent molecule in the act of splitting; the Y-shape characteristic of the replication process is clearly imaged.

Scanning tunneling microscopy is emerging as a very promising technique for studying biological specimens with atomic-scale resolution. The technique is particularly suitable for small-size molecular structures, since the small size reduces the problems due to electrical insulation. Among those, DNA is by far the most interesting. Other authors have reported STM images of uncoated DNA molecules, either in water² or in air.³⁻⁸ Most of such studies were performed on unlabeled DNA. In the present case, images were obtained on uncoated and unlabelled DNA in air, thereby reproducing the excellent experimental conditions of Ref. 3.

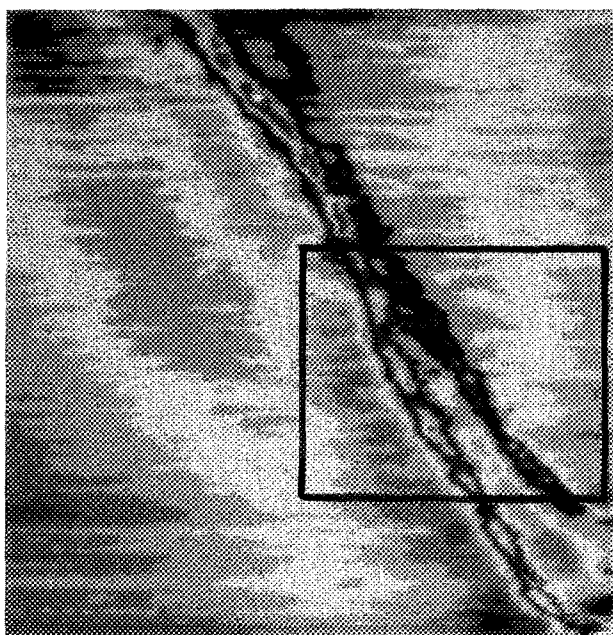
Highly polymerized DNA extracted from salmon (Sigma D-1626), suspended in a 4% phosphate buffered saline solution (PBSS), was deposited on atomically flat substrates obtained by cleaving highly oriented pyrolytic graphite (HOPG). After deposition, the DNA-containing solution drops were mechanically spread on the substrate and then dried in an oven at 50 °C for 2 h. This approach produces DNA specimens suitable for STM imaging without metal coating or labeling.

The preparation technique and the STM methodology were tested by performing experiments on a number of specimens. We confirmed many of the empirical findings of Ref. 3. For example, we did find that the bias can produce a significant interaction between tip and specimen, thereby altering the observed molecules between subsequent scans. We also found that periodic structures can be simulated by the

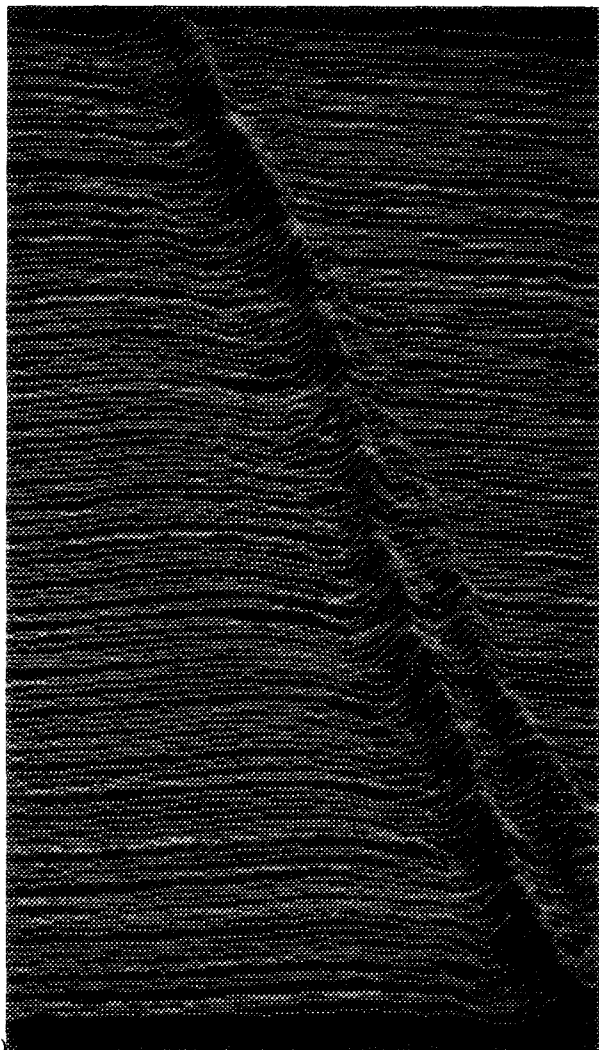
graphite surface after deposition of DNA-free saline solution. Such structures, however, have periodicities one order of magnitude larger than DNA. In order to rule out spurious substrate effects in the images discussed here, we examined approximately 500 images from DNA-free regions, never finding structure that could be confused with DNA. We agree, therefore, with Ref. 3, and find that graphite substrates are much better than polished metal substrates in producing high-quality images. We empirically found that the thermal treatment of the specimen during dehydration is extremely important in producing stable DNA molecules on graphite substrates.

The replicating DNA molecule is shown by the STM images of Figs. 1 and 2. These images were produced at atmospheric pressure in the topographic mode, using a modified McAllister scanning tunneling microscope with a gold tip. Prior to taking micrographs of the DNA molecule, the STMs performance was checked by imaging the graphite substrate with atomic-scale resolution. Figure 1(a) shows a $438 \times 436 \text{ \AA}^2$ area, with simulated colors; Fig. 1(b) presents the same image with a three-dimensional plot. Figure 2(a) presents a grey-scale image of the small area in Fig. 1(a), $221 \times 177 \text{ \AA}^2$, and Fig. 2(b) a three-dimensional plot of a slightly larger area, $221 \times 221 \text{ \AA}^2$. The images are part of a set including a total of 27 micrographs; each part of the same molecule was imaged several times over a period of hours, demonstrating the excellent reproducibility of the experiment and the stability of the specimen. In particular, we found no evidence of the tip-specimen interaction effects that were previously mentioned (Ref. 3).

From Figs. 1 and 2, one observes the typical periodicity related to the DNAs double helix. This periodicity is emphasized by the line height profile of Fig. 2(c), derived from the data of Fig. 2(a) for the dashed line following the axis of the DNA branch on the left-hand side. We estimate a period of approximately 44 Å. This is well within the range of periodicity values reported by other authors, and in particular by

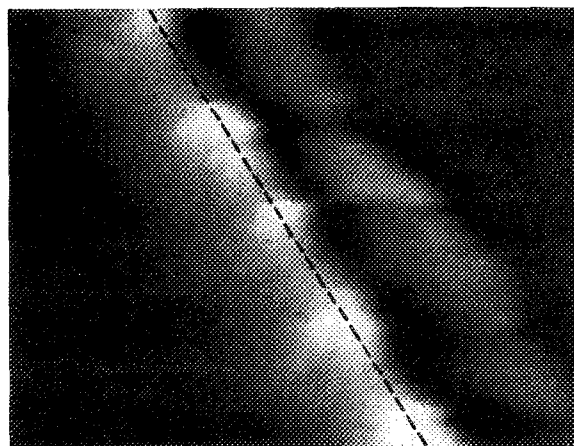


(a)

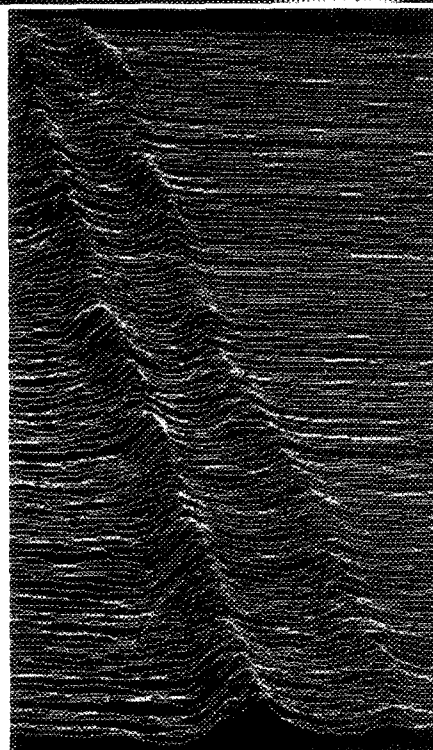


(b)

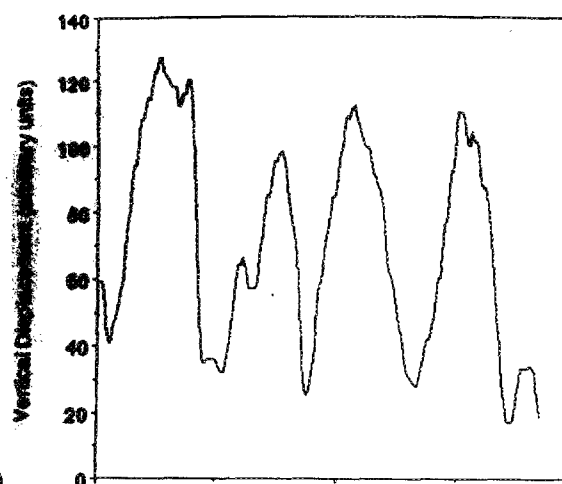
FIG. 1. (a) Scanning tunneling topographic micrograph of a DNA molecule in the act of replication, shown with simulated colors; the small area is shown enlarged in Fig. 2(a); (b) the same image shown with three-dimensional plot. The tunneling current was 3.0 nA, the sample bias voltage 35 mV, the tip velocity 550 Å/s, and the area was $438 \times 436 \text{ \AA}^2$.



(a)



(b)



(c)

FIG. 2. (a) Enlarged detail of the small area in Fig. 1(a), shown with a greyscale image. The tunneling current was 3.0 nA, the sample bias voltage 35 mV, the tip velocity 440 Å/s, and the area was $221 \times 177 \text{ \AA}^2$. (b) three-dimensional plot of an area slightly more extended than that of (a), $221 \times 221 \text{ \AA}^2$. (c) Line height profile from the data of (a), taken along the axis of the left-hand side branch (dashed line). Note the periodicity, corresponding to a helix pitch of approximately 44 Å.

the authors of Ref. 3, whose experimental conditions are quite similar to ours. Different periods, still similar to those of Ref. 3, were found in other specimens in the course of our study; such periods are consistently larger than those found for crystalline DNA. This increase in the helix pitch with respect to crystalline DNA has been tentatively attributed to intercalation of salts from the saline solution.³

The Y structure and the two branches of the replicating molecule is clearly visible in Figs. 1 and 2 as well as in a series of other images. Such images were taken to carefully examine the bifurcation region and rule out spurious causes of the observed Y structure, such as two overlapping DNA molecules. These tests leave a single replicating DNA molecule as the only plausible interpretation of the observed images. Spurious substrate effects were ruled out by the previously mentioned, extensive tests on DNA-free regions. Note, in particular, that the size of the right-hand side branch is generally smaller than that of the left-hand side branch, suggesting that the right-hand side branch is a single strand. This is consistent with the fact that the centrifugation during the DNA isolation process removes proteins and enzymes. In fact, during replication the two strands of the parent molecule separate and the enzyme catalyzes the synthesis of two new strands complementary to the parent strands. This synthesis occurs in triplets, monotonically in one direction for one of the two new strands, and back and forth for the other. Therefore, the enzyme is continuously attached to the first of the two new strands, but only for a fraction of the time to the second of the new strands. During centrifugation, the new strand that is continuously attached to the enzyme is removed together with the enzyme, thus leaving the single (parent) strand that appears in the right-hand side of the image.

These results are not only important because of the intrin-

sic interest of directly observing the replication of DNA—but also because they confirm the finding of other authors, that DNA in a nearly natural state is imageable with the STM technique. These results, therefore, open the way to an extensive use of STM studies in molecular biology problems such as the direct investigation of the local molecular structure of a single gene. Preliminary tests along these lines are currently underway.

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