ISSN 0026-8933, Molecular Biology, 2008, Vol. 42, No. 3, pp. 469–477. © Pleiades Publishing, Inc., 2008.
Original Russian Text © O.Yu. Limanskaya, A.P. Limanskii, 2008, published in Molekulyarnaya Biologiya, 2008, Vol. 42, No. 3, pp. 533–542.

STRUCTURAL–FUNCTIONAL ANALYSIS OF BIOPOLYMERS AND THEIR COMPLEXES

UDC 577.2:577.32

Imaging of T7 RNA Polymerase Elongation Complexes by Atomic Force Microscopy

O. Yu. Limanskaya[a, c] and A. P. Limanskii[a, b]

[a] *Mechnikov Institute of Microbiology and Immunology, Academy of Medical Sciences of Ukraine, Kharkov, 61057 Ukraine; e-mail: o.lymunskiy@mail.ru*

[b] *Laboratory of Plasma Membrane and Nuclear Signaling, Graduate School of Biostudies, Kyoto University, Kyoto, 606-8502 Japan*

[c] *Institute of Experimental and Clinical Veterinary Medicine, Kharkov, 61023 Ukraine*

Received August 23, 2007
Accepted for publication December 26, 2007

Abstract—Atomic force microscopy was used to visualize the complexes of bacteriophage T7 RNA polymerase (RNAP) with a DNA template during transcription. A 1414-bp fragment of linearized pGEMEX DNA was used as a template; the fragment contained the T7 promoter and terminator asymmetrically located at the template ends. Images of stable complexes of T7 RNAP and template terminal fragments were obtained for single molecules. Individual template DNA molecules bound with two or three T7 RNAP molecules, which corresponded to transcription initiation, elongation, and termination, and complexes containing RNA transcripts were imaged under conditions preventing nonspecific binding. The results suggest that T7 RNAP binds initially to the DNA template terminal fragment located near the promoter in order to exclude skipping the promoter site.

DOI: 10.1134/S0026893308030175

Key words: atomic force microscopy, transcription, T7 RNA polymerase, RNA transcript

INTRODUCTION

Transcription, an important stage of gene expression and its regulation, proceeds via three steps—initiation, elongation, and termination. The advance in high-resolution microscopy has made it possible to obtain new data on the structure of elongation complexes.

Electron microscopy and atomic force microscopy (AFM) have yielded important information about the spatial arrangement of DNA, RNA, and RNA polymerase (RNAP) during elongation, namely, about the global topology of the open promoter and elongation complex. Experimental studies of eukaryotic and prokaryotic transcription have made it possible to propose a model for the elongation complex, implying DNA wrapping around the enzyme [1, 2]. In the elongation complex, DNA wrapping around RNAP and the formation of an RNA–DNA hybrid induce an opening of the DNA double helix and the formation of a transcription loop. However, it is yet unclear how RNAP synthesizes long DNA fragments. The RNAP movement along DNA in vivo has especially caused many discussions, including the question whether RNAP moves around DNA or is in such a rotationally strained state that DNA has to rotate around the helix axis so that a DNA strand passes through the protein. Another intriguing problem is imaging of growing RNA molecules during elongation.

Note that a high-resolution AFM imaging of protein–nucleic acid complexes makes it possible to directly study the biological structures under near physiological conditions (in particular, without crystallization). Unlike the traditional microscopy methods, providing averaged information about a group of molecules, AFM gives the data on the structure and functioning of individual molecules. The only requirement for an AFM examination of the biomacromolecular surface topography is that the molecules be adsorbed on a flat substrate. Thus, many papers report the AFM imaging of protein (RNAP included) complexes with DNA [1, 3].

An important feature of the elongation complexes formed during transcription (DNA–RNAP–RNA transcript) is that the elongation complex rapidly dissociates upon transcription completion. The dissociation rate for phage T7 RNAP is considerably higher than that for eukaryotic RNAP. This fact essentially hinders the imaging of both the overall elongation complex and individual RNA transcripts. Therefore, DNA templates synthesized so that transcription is stopped at certain positions [1] are frequently used for the AFM imaging of elongation complexes. In this work, we used another approach to prevent rapid dissocia-

tion of the elongation complex; namely, our DNA template contained the T7 RNAP transcription terminator 1122 bp away from the transcription initiation site. The termination efficiency is controllable, as it depends on the concentrations of reaction mixture components, ionic strength, and transcription buffer pH and amounts to 70–80%.

In this work, we optimized the in vitro transcription conditions for further AFM imaging of the complex formed by single-subunit T7 RNAP with a linear DNA containing the promoter and terminator for this enzyme. Several factors influence the transcription efficiency, namely, the reaction temperature and time, the concentrations of the components, and the ionic strength. We imaged the T7 RNAP–DNA complexes after transcription performed at various temperatures for various times by using several transcription buffers differing in the composition and concentrations of components.

Images of both the terminal (formed by T7 RNAP with DNA template terminal fragments) and specific complexes (first and foremost, with the promoter) formed by T7 RNAP with individual DNA template molecules were obtained.

EXPERIMENTAL

Transcription. A 1414-bp amplicon containing promoter A1 (one of the 17 known promoters of the bacteriophage T7 genome) and the T7 RNAP terminator was used as a template. To obtain the DNA template, 3993-bp supercoiled pGEMEX (Promega, United States) was digested with *Sca*I and amplified and the amplicon was purified. Transcription was performed according to the below protocols using a T7 RNAP transcription kit (Promega, United States), a MegaScript T7 (Ambion, United States) kit, and a New England Biolabs (United Kingdom) kit at various temperatures for different times.

The DNA template for transcription was designed so that it contained the T7 RNAP promoter and terminator located asymmetrically at the ends of the 1414-bp amplicon: the promoter was at positions 1212–1231 (i.e., 200 bp from the 3' end of the template DNA strand) and the terminator was at positions 91–182 (i.e., 90 bp from the 5' end of the template DNA strand).

Three transcription buffers were used, namely, buffer A (Promega, United States), buffer B (Ambion, United States), and buffer C [1]. Buffer A contained 40 mM Tris-HCl (pH 7.9), 6 mM $MgCl_2$, 10 mM NaCl, 10 mM DTT, 2 mM spermidine, 0.05% Tween 20, 40 units of RNasine, and 20 units of RNAP in a total volume of 20 μl. Transcription was initiated by adding CTP, GTP, UTP, and ATP to a final concentration of 200 μM.

Buffer B contained the transcription mixture (Ambion, United States), 40 units of RNasine, 20 units of RNAP, and 20 μM CTP, GTP, UTP, and ATP in a total volume of 20 μl. After 20- to 65-min incubation at 31°C, the reaction was stopped by heating at 70°C for 15 min.

Buffer C contained 20 mM Tris-HCl (pH 7.9), 5 mM $MgCl_2$, 50 mM KCl, 1 mM DTT, 40 units of RNasine, and 20 units of RNAP in a total volume of 20 μl. Transcription was initiated by adding CTP, GTP, UTP, and ATP to a final concentration of 100 μM. After 65-min incubation at 31°C, the reaction was stopped by quick chilling at 0°C.

After transcription, the reaction mixture was supplemented with 1 μl of RNase-free DNase I (Ambion, United States) and incubated for 15 min at 37°C to remove the DNA template and degraded DNA, which could contaminate the RNA preparation. To inactivate DNase, the reaction mixture was incubated at 70°C for 10 min. The transcription efficiency was checked by electrophoresis in 1.2% agarose gel containing 1.8% formaldehyde. Transcription was performed in a specialized room for RNA handling; ultrapure water treated with diethyl dicarbonate to inhibit RNases was used.

The corresponding bands in the pattern after denaturing electrophoresis of the transcription products obtained with T7 RNAP indicated that RNA transcripts of the expected lengths were synthesized.

For denaturing electrophoresis, the reaction mixture was supplemented with an equal volume of a sample application buffer (90% formamide, 0.01% Xylene Cyanol, 0.01% Bromphenol Blue, 10 mM EDTA, 0.01% SDS). The sizes of RNA transcripts synthesized on pGEMEX were assessed using a control DNA template, pTRI-Xef, from a MegaScript T7 transcription kit (Ambion, United States). Transcription performed according to the manufacturer's protocol gave only fill-sized RNA transcripts of 1890 bp; their size was assessed using G 319A RNA markers (Promega, United States).

AFM. A Nanoscope IV MultiMode System microscope (Veeco Instruments, United States) equipped with an E scanner was used in the work. AFM images of DNA were acquired using a vibrating AFM variant in the air in a height mode, using OMCL-AC160TS cantilevers (Olympus Optical, Japan) with a resonant frequency of 340–360 kHz and a spring constant of 42 N/m. The resulting images of 512 × 512 pixels were smoothed and analyzed using the Nanoscope (v. 5.12r3) software (Veeco Instruments, United States).

Visualization of T7 RNAP–DNA complexes. Biomolecules were immobilized on freshly cleaved mica from a buffer containing magnesium ions. The AFM images of DNA template molecules adsorbed on

mica were used as a control; these molecules displayed an unstretched shape and smooth borders, and their fragments were uniformly immobilized on the substrate surface.

Preparation of DNA samples for PCR and T7 RNAP–DNA complexes for AFM. For the polymerase chain reaction (PCR), we designed primers L1 and L2, which flanked the pGEMEX DNA fragment containing the T7 RNAP promoter and terminator. Forward primer L1 (5'-cgc tta caa ttt cca ttc gcc att c-3', 3748–3772; the position is as in circular pGEMEX) and reverse primer L2 (5'-ctg att ctg tgg ata acc gta tta ccg-3', 1168–1142) were obtained from Sigma (Japan).

Hot start PCR was performed in 50 μl of the reaction mixture in a GeneAmp 9700 thermal cycler (Perkin Elmer, United States), using the following mode: initial incubation at 95°C for 2 min and 35 cycles of denaturation at 95°C for 1 min, annealing at 71°C for 1 min, and synthesis at 74°C, for 1 min. The annealing temperature was calculated using the Oligo program [4].

The amplicons were visualized by separating 15 μl of the PCR product in 2% agarose gel with subsequent ethidium bromide staining.

The amplified DNA fragment was cut off from gel under illumination with a long-wavelength low-intensity UV radiation source (Bio-Rad, United States) and purified from nucleotides, primers, DNA polymerase, and ethidium bromide with a QIAquick PCR purification kit as recommended by QIAgen (Japan); the target DNA fragment was extracted with phenol–chloroform with subsequent ethanol precipitation.

Two types of high-fidelity DNA polymerase was used for PCR, namely, Pyrobest DNA polymerase (TaKaRa, Japan) and Platinum DNA polymerase (Invitrogen, Japan).

The T7 RNAP–DNA complex was immobilized onto freshly cleaved mica in 10 mM HEPES containing 2.5 mM $MgCl_2$ or the transcription buffer, as the latter also contained magnesium ions. A 10-μl drop of a solution containing the complex with a T7 RNAP-to-DNA molar ratio varying from 1 to 100 in TE (10 mM Tris-HCl, pH 7.9, 1 mM EDTA) was placed onto a mica fragment with a size of 1 cm^2, washed with ultrapure water after a 2-min exposure, blown with nitrogen, and immediately used for visualization.

The T7 RNAP concentration in the stock solution was determined spectrophotometrically and amounted to 6.6×10^{-6} M T7 RNAP (Promega). The concentration of linear pGEMEX DNA (amplicon), used as a template for transcription, was 200–800 pM. The concentration of the purified PCR product in the stock solution was measured spectrophotometrically. Complexes were obtained by adding a T7 RNAP solution to an equal volume of the DNA template, which was prepared by successive dilutions of the amplicon stock solution. When imaging the T7 RNAP–DNA complex, the ratio of T7 RNAP to DNA was varied from 1 to 100. The corresponding T7 RNAP solutions were prepared by successive dilutions of the stock solution.

RESULTS AND DISCUSSION

The AFM images of the DNA template or amplicon immobilized on freshly cleaved mica in HEPES or the DNA–T7 RNAP complex without performing transcription were used as a control. In the absence of T7 RNAP, bends and loops of linear pGEMEX DNA molecules were undetectable in AFM images. An important feature of AFM is that it is possible to directly measure the contour length of DNA molecules with a sufficiently high resolution with the help of the corresponding software. Note that measurement of the DNA contour length was among the first AFM applications [5]. Using the plotted Gaussian distribution of the amplicon contour lengths measured directly in AFM images, we determined the contour length of the DNA template under various conditions. In particular, the contour length of the DNA molecules immobilized on mica from buffer A, containing divalent magnesium cations and trivalent spermidine cations, amounted to 480 ± 20 nm. This value fits well the theoretically calculated length of the 1414-bp DNA template, 480 nm in the assumption of its B form ($L = 1414$ bp $\times 0.34$ nm).

After adding T7 RNAP to the transcription mixture with the DNA template, elongation complexes were formed during transcription and displayed the bends characteristic of DNA–protein complexes [1, 6, 7]. It is evident in the AFM images shown in Figs. 1a and 1b that DNA molecules 2 and 3 formed complexes with only one T7 RNAP molecule, while molecule 1 and the molecules shown in Figs. 1c and 1d bound three T7 RNAP molecules. The contour length of the DNA template bound with one T7 RNAP molecule in transcription buffer A (in the absence of transcription) amounted to 456 ± 13 nm. As for the DNA template that bound two T7 RNAP molecules, its contour length decreased to 448 ± 30 nm.

The decreased DNA contour length in complex with T7 RNAP is explainable by DNA wrapping around the RNAP molecule. The corresponding model was proposed by Studitsky et al. [8]. DNA wrapping around an RNAP molecule must decrease the contour length, as was demonstrated with *Escherichia coli* RNAP [1]. The elongation complexes of RNAP and a DNA template were visualized by AFM after a transcription arrest at certain sites, and the DNA molecule was actually wrapped around the RNAP molecule [1].

On the other hand, the DNA–T7 RNAP complexes of the molecules shown in Figs. 1a–1c differed qualitatively from the complexes of the molecule in Fig. 1d, whose binding was accompanied by charac-

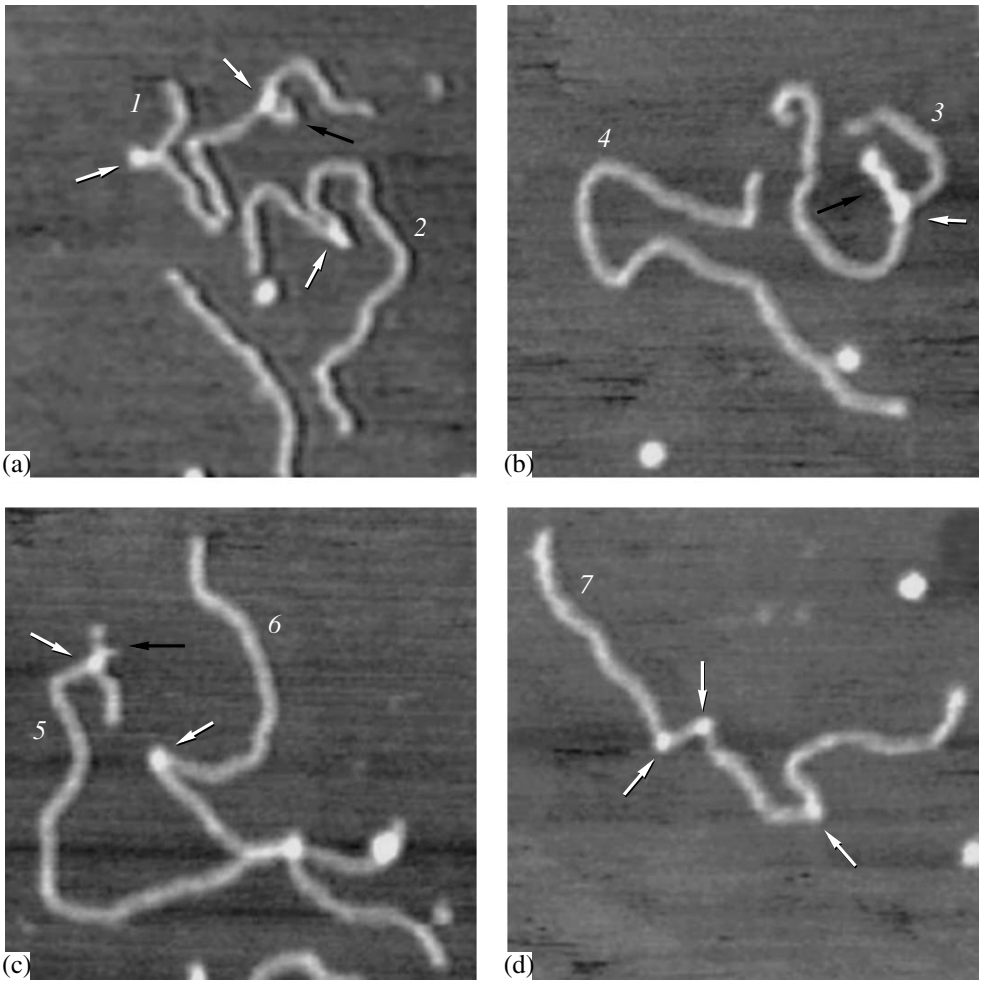

Fig. 1. High-resolution AFM images of T7 RNAP–DNA complexes. T7 RNAP molecules look like spheres with a diameter of 8 nm. White arrows indicate the complexes formed by T7 RNAP and DNA molecules and black arrows indicate (a, c) an RNA transcript and (b) a twisted fragment of the DNA template. Transcription was performed in buffer A at room temperature for 60 min, and the reaction mixture was immediately used for AFM imaging. Scan size: (a) 348 × 348, (b) 306 × 306, (c) 298 × 298, and (d) 299 × 299 nm. Contour lengths of molecules: (a) 445–450 and (b) 454–457 nm.

teristic bending. Black arrows in Figs. 1a–1c indicate the fragments that can be interpreted as RNA transcripts (note that, despite a comprehensive analysis of the similar-sized AFM images shown in [1], we failed to find any DNA–RNAP complex containing a growing RNA transcript). Nonetheless, a thorough analysis of the AFM image of molecule 3 (Fig. 1b) with a higher resolution, construction of cross-sections of the fragments (Fig. 2), and their reconstructed 3D images (Fig. 3) suggest that the indicated fragment of molecule 3 (Fig. 1b) can be a fragment of the DNA template.

Note that the height of DNA molecule adsorbed on the mica surface and measured using AFM amounted to 0.3–0.5 nm. In an AFM image, T7 RNAP molecules (98.8 kDa) looked like spheres with a diameter of about 8 nm. The height of the DNA–T7 RNAP complex equaled from 0.6 nm (Figs. 2a, 2b) to 1 nm (Figs. 2c, 2d). The length of molecule 3 with a wound fragment (indicated with a black arrow in Fig. 1b) was 457 nm, thereby suggesting that the imaged fragment was part of the DNA template rather than an RNA transcript. In addition, the height of the knots formed by twisted double-stranded DNA amounted to 0.8 nm (Fig. 2d), also fitting the height of the knots of twisted DNA immobilized on mica [9].

It is important that both specific and nonspecific complexes are formed as a result of the interaction between DNA and T7 RNAP molecules. Specific binding (interaction with the promoter) is relatively insensitive to changes in ionic strength but depends on the conformation of the DNA fragment. Nonspecific DNA–T7 RNAP complexes are formed due to electrostatic interactions between positively charged RNAP residues and negatively charged DNA phosphate groups. Nonspecific binding is extremely sensitive to

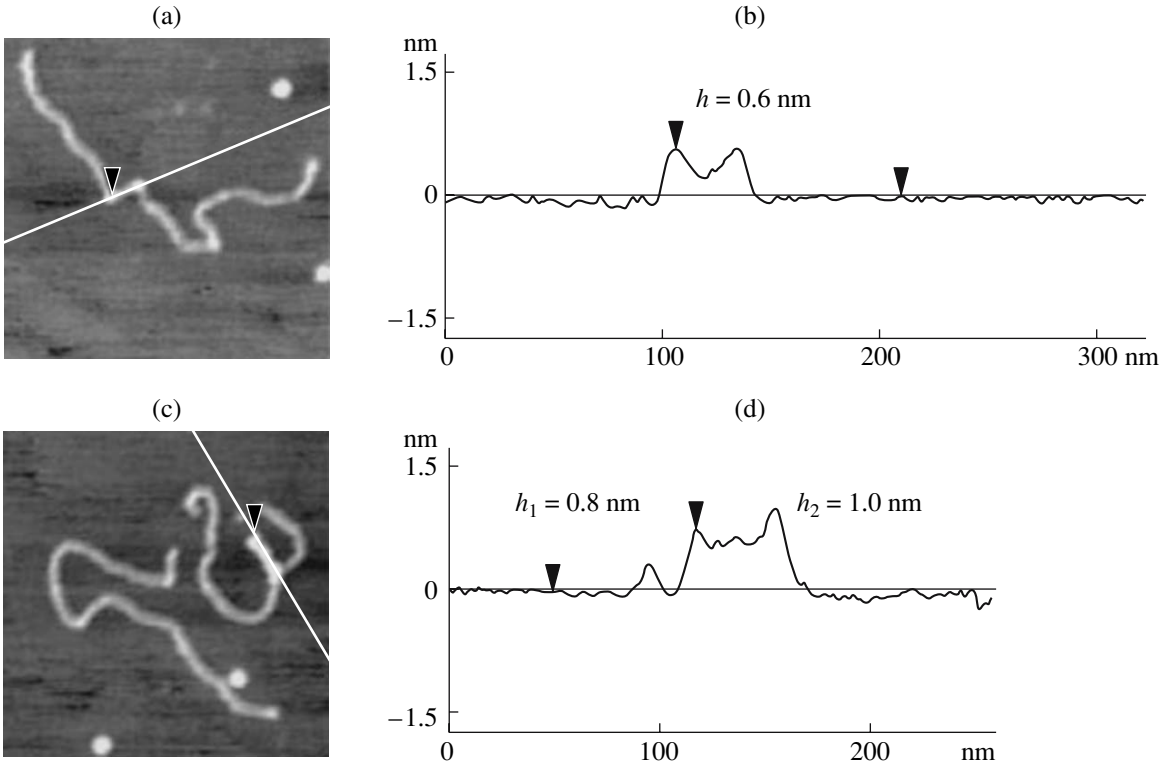

Fig. 2. (a, c) AFM images of pGEMEX DNA–T7 RNAP complexes and (b, d) the corresponding longitudinal cross-sections. The line of the secant plane perpendicular to the image plane is shown (a, c). Peaks on the cross-sections correspond to the maximal height of complexes or molecules. (b) The height h of both pGEMEX DNA–T7 RNAP complexes amounts to 0.6 nm. (d) h_1 is the height of the pGEMEX DNA–T7 RNAP complex and h_2 is the maximal height of the twisted pGEMEX DNA fragment.

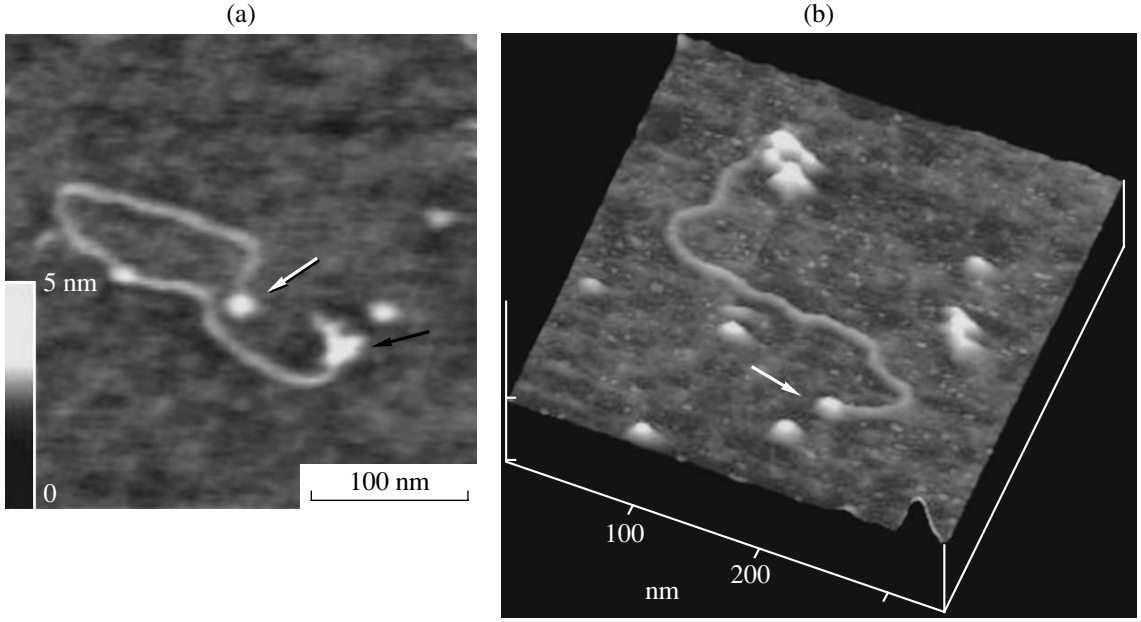

Fig. 3. (a) AFM and (b) 3D images of the complexes formed by T7 RNAP molecules with the terminal fragments of the DNA template. The white arrow denotes the initiation complex and the black arrow indicates the complex in the transcription terminator region. (a) Scan size is 297 × 297 nm. The gray scale corresponds to the height range from 0 to 5 nm. Transcription was performed in buffer B at 31°C for 65 min.

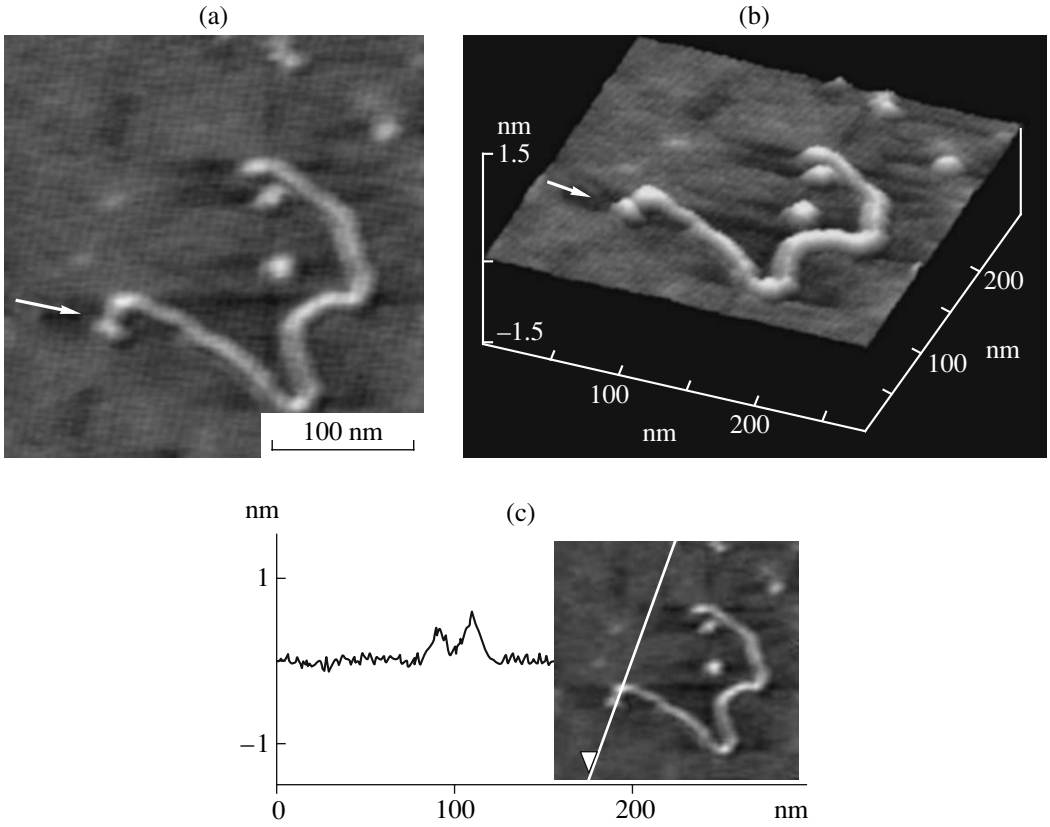

Fig. 4. Image of a single linear 1414-bp DNA molecule in complex with T7 RNAP (indicated with an arrow) after transcription in buffer A at 37°C for 4 min. T7 RNAP molecules look like spheres with a diameter of about 8 nm. (a) An AFM image with the scan size 280 × 280nm, (b) a 3D image, and (c) cross-section of the T7 RNAP–DNA complex used to determine the heights of DNA and T7 RNAP molecules. The inset shows the line of the secant plane perpendicular to the image plane. The height of the T7 RNAP molecule within the T7 RNAP–DNA complex amounts to 0.6 nm and that of the DNA molecule is 0.4 nm.

the ionic strength of the reaction mixture but is insensitive to the degree of DNA supercoiling. Analyzing the properties of nonspecific and specific DNA–RNAP complexes, Smeekens and Romano [10] inferred that the main components of the mechanism regulating T7 transcription are the efficiency and rate of the formation of the open RNAP–promoter complex rather than binding to different promoters contained in the T7 genome.

In this work, we used the promoter located at positions 1212–1231 of the DNA template, i.e., at a distance of 200 nt from the amplicon end, which corresponds to ~70 nm (200 nt × 0.34 nm). Note AFM allows a reliable imaging of the complex formed at such a distance from the amplicon end. On the other hand, of a rather large set of the DNA–T7 RNAP complexes analyzed (over 200), about 30 complexes of RNAP with the terminal fragments were imaged. Typical AFM and 3D images of such complexes are shown in Figs. 3 and 4.

Let us consider the imaged complexes formed by T7 RNAP at both ends of the amplicon in more detail. When transcription was performed at 37°C for 4 min, complexes of only one type were detected, with T7 RNAP attached to one of the ends of the DNA template (Fig. 4). The height measured for this complex (Fig. 4a, arrow) directly in an AFM image with the help of constructed cross-sections (Fig. 4c) was ~0.5 nm, which fits the height of a single T7 RNAP molecule. However, two types of complexes located at both ends of the DNA template were imaged in the case of transcription at 31°C for 65 min (Fig. 3), namely, T7 RNAP complexes with the promoter and terminator. The height of the other T7 RNAP–DNA complex (Figs. 3a, b; arrows) was 0.93–1.00 nm.

It is known that a decrease in transcription temperature from 37 to 30°C increases in the content of full-sized RNA transcripts. On the other hand, a rather high NaCl concentration (>30 mM) can decrease the amount of RNA transcripts because of DNA template precipitation [11]. Note that the ionic strength (**I**) of the buffers used for transcription amounted to 70–80 mM Na^+.

Transcription can be considered as a scanning of the DNA template by RNAP. The scanning direction is

specified by the promoter sequence in the template DNA strand [12]. However, we believe that, to search for a promoter and to avoid the possibility of skipping this site, the precision "machine," such as RNAP, starts the scanning from one of the terminal DNA fragments. Thus, we explain a rather large number of imaged terminal complexes, or the complexes that precede the transcription initiation complexes, by this particular fact. Our assumptions agree with the experimental results of AFM imaging of RNAP diffusion during a search for a promoter [13] and the model of protein movement along DNA (a quick transfer of the protein between different DNA fragments via one-dimensional diffusion and weak nonspecific binding) [14].

The transcription terminator was at the 5' end of the template strand of the amplicon at a distance of ~90 bp from its end. When the elongation complex (DNA–RNAP–RNA transcript) reaches the terminator, it stops and dissociates. However, we imaged several DNA molecules that formed a complex with T7 RNAP at both ends of the template (Fig. 3). Moreover, three T7 RNAP molecules bound to DNA are seen in Fig. 3a: one formed the initiation complex (white arrow), the second formed a complex in the terminator region (black arrow), and the third was in the middle of the DNA template. The presence of several T7 RNAP molecules bound to one DNA molecule confirms that, after one T7 RNAP molecule has started transcription, another enzyme molecule can bind to the terminal fragment of the DNA template to commence the next round of transcription preinitiation and elongation.

Several T7 RNAP molecules could be bound to the DNA template at the terminator region (Figs. 3a, 3b; black arrow). In addition to the terminal T7 RNAP–DNA complexes, five more RNAP molecules that did not form a complex with the DNA template are seen in the 3D image (Fig. 3b). The imaged terminal complex (Figs. 3a, 3b; white arrow) was specific, as transcription was performed with excess T7 RNAP and at a rather high ionic strength (70–80 mM Na^+), preventing a nonspecific binding of T7 RNAP to DNA, and only one T7 RNAP–DNA complex formed under these conditions. Analogous T7 RNAP–DNA complexes have been imaged by Crampton et al. [15]. However, we consider that they were erroneously interpreted as nonspecific.

The presence of several T7 RNAP molecules bound to the DNA template during transcription initiation and elongation (Fig. 3a) demonstrates that several molecules, rather than a single molecule, initiate transcription from one promoter. This finding complies with the data of Epstein and Nudler [16], who demonstrated the cooperation between RNAP molecules in transcription elongation. It is known that transcription in bacteria and eukaryotes proceeds at a high rate despite numerous transcription blocks. As has been demonstrated, the majority of internal and external transcription blocks (pause and stop signals) disappear when more than one RNAP molecule initiate transcription from the same promoter. On the contrary, the presence of internal terminators has an essential effect on the elongation rate and the yield of full-size transcripts in the case of a single transcription cycle.

Kinetic analysis of the transcription initiation and elongation for single T7 RNAP molecules has demonstrated that the duration of the initiation stage is 4 s and, consequently, about 30 s are necessary for synthesizing an RNA transcript of 1200 nt [17]. Therefore, 4-min transcription was sufficient for synthesizing RNA transcripts on the 1414-bp DNA template used. However, only complexes with one end of the DNA template, similar to the variant shown in Fig. 4, were imaged after 4-min transcription (in buffer A). When the transcription time was extended to 65 min, we detected the T7 RNAP complexes with both terminal fragments of the DNA template (Fig. 3). We believe that the complex indicated with a black arrow in Fig. 3 corresponds to the complex formed by T7 RNAP with the terminator on the DNA template. Presumably, several T7 RNAP molecules with the corresponding RNA transcripts stop one after another at the terminator after completing elongation. This is suggested by the large size of the complex and the presence of a T7 RNAP molecule inside the DNA template simultaneously with the complexes at both terminal sites of the DNA template. According to our opinion, a decrease in the dissociation rate of the elongation complexes that reached the terminator at a lower transcription temperature (31°C versus 37°C) makes it possible to image the complexes at the termination site.

Among a rather large set of DNA–T7 RNAP complexes analyzed (over 200), about 30 complexes of T7 RNAP with the terminal DNA fragments were imaged. Several circumstances can explain the fact that we imaged a rather large number of T7 RNAP complexes with the terminal fragments of the DNA template. First, both the promoter and terminator are at the ends of the DNA template. As the binding constant of T7 RNAP with a highly specific promoter is considerably higher than the nonspecific binding constant (for any site of the DNA template) [10], the promoter–T7 RNAP complex under conditions of T7 RNAP–DNA dissociation is the most stable of all complexes formed during transcription initiation and termination. Second, the dissociation time of the T7 RNAP–promoter complex is considerably longer than the time required for a transition to the elongation complex; therefore, other conditions being equal, the probability to image the complex with the promoter is rather high. Third, the enzyme is paused and stopped

at the terminator, thereby statistically increasing the probability of imaging the complexes with terminal fragments of the DNA template. On the other hand, the fact that the elongation complexes were imaged at the transcription termination site only in the case of transcription at 31°C (Fig. 3) suggests that the dissociation rate of elongation complexes reaching the terminator under these conditions decreases due to the lower temperature of transcription.

To directly image the RNA transcripts, the DNA template and primers were removed by incubating the reaction mixture with DNase I after transcription. Using high-resolution AFM images, we determined that RNA transcripts immobilized on mica formed rodlike condensed structures with a length of 122 ± 10 nm and a length-to-width ratio of 4.5–5.0. As this length is considerably shorter than the expected transcript length, equaling 336 nm (1122 nt × 0.3 nm = 336 nm), we can infer that single-stranded RNA molecules formed multistranded condensed structures. It has been earlier demonstrated by AFM that RNA molecules synthesized from a linear DNA template form such condensed structures with a length amounting to half of the DNA template length [18].

Analysis of the images of RNA transcripts and supercoiled DNA molecules immobilized on mica with various hydrophobicities and surface charge densities has demonstrated that the hydrophobicity and surface cation density of mica used to immobilize RNA transcripts are too high for imaging linear single-stranded RNA molecules but not the condensed structures formed by such molecules [9, 19]. Therefore, the imaging of individual RNA molecules requires optimization of the mica surface cation density so that RNA molecules immobilized on mica would form hairpins or would be stretched rather than forming condensed structures.

Thus, we used AFM to image the complexes formed by T7 RNAP with a DNA template during transcription elongation. Under conditions inhibiting nonspecific binding, T7 RNAP molecules can simultaneously form complexes with both the terminal fragments and the terminator on one DNA template molecule (containing both the promoter and terminator for T7 RNAP) during transcription elongation. When imaging the elongation and transcription termination complexes, room temperature or a temperature of 31°C is optimal for transcription. We believe that further experiments utilizing the AFM capabilities (imaging directly in a buffer and measuring the molecular forces at the level of individual pairs of molecules) will provide more detailed information about the transcription elongation complexes.

ACKNOWLEDGMENTS

We are grateful to A.V. Sivolob (Shevchenko Kiev National University, Kiev, Ukraine) for his valuable criticism during manuscript preparation.

The work was supported by the International Center of Science and Technology (Japan) and the Academy of Medical Sciences of Ukraine (grant no. AMN 72.2007).

REFERENCES

1. Rivetti C., Codeluppi S., Dieci G., Bustamante C. 2003. Visualizing RNA extrusion and DNA wrapping in transcription elongation complexes of bacterial and eukaryotic RNA polymerases. *J. Mol. Biol.* **326**, 1413–1426.
2. Walter W., Studitsky V. 2004. Construction, analysis, and transcription of model nucleosomal templates. *Methods*. **33**, 18–24.
3. Guthold M., Bezanilla M., Erie D., Jenkins B., Hansma H., Bustamante C. 1994. Following the assembly of RNA polymerase–DNA complexes in aqueous solutions with the scanning force microscopy. *Proc. Natl. Acad. Sci. USA*. **91**, 12927–12930.
4. Rychlik W., Spencer W.J., Rhoads R.E. 1990. Optimization of the annealing temperature for DNA amplification in vitro. *Nucleic Acids Res*. **18**, 6409–6417.
5. Lyubchenko Y., Jacobs B., Lindsay S. 1992. Atomic force microscopy of reovirus dsRNA: A routine technique for length measurement. *Nucleic Acids Res*. **20**, 3983–3986.
6. Rees W., Keller R., Vesenka J., Yang C., Bustamante C. 1993. Evidence of DNA bending in transcription complexes imaged by scanning force microscopy. *Science*. **260**, 1646–1649.
7. Kosikov K., Gorin A., Lue X., Olson W., Manning G. 2002. Bending of DNA by asymmetric charge neutralization: All-atom energy simulations. *J. Am. Chem. Soc*. **124**, 4838–4847.
8. Studitsky V., Walter W., Kireeva M., Kashlev M., Filsenfeld G. 2004. Chromatin remodeling by RNA polymerases. *Trends Biochem. Biosci*. **29**, 127–135.
9. Limanskaya L.A., Limansky A.P. 2006. S-DNA, oversupercoiled DNA with a 1.94- to 2.19-Å rise per base pair. *Mol. Biol*. **40**, 122–136.
10. Smeekens S., Romano L. 1986. Promoter and nonspecific DNA binding by the T7 RNA polymerase. *Nucleic Acids Research*. **14**, 2812–2827.
11. Komissarova N., Becker J., Solter S., Kireeva M., Kashlev M. 2002. Shortening of RNA:DNA hybrid in the elongation complex of RNA polymerase is a prerequisite for transcription termination. *Mol. Cell*. **10**, 1151–1162.
12. Milligan J., Groebe D., Witherell G., Uhlenbeck O. 1987. Oligonucleotide synthesis using T7 RNA polymerase and synthetic DNA template. *Nucleic Acids Res*. **15**, 8783–8798.
13. Guthold M., Zhu X., Rivetti C. 1999. Direct observation of one-dimensional diffusion and transcription by

Escherichia coli RNA polymerase. *Biophys. J.* **77**, 2284–2294.

14. Berg O., Winter R., Von Hippel P. 1981. Diffusion-driven mechanisms of protein translocation on nucleic acids: 1. Models and theory. *Biochemistry*. **20**, 6929–6948.
15. Crampton N., Thomson N., Kirkham J., Gibson C., Bonass W. 2006. Imaging RNA polymerase–amelogenin gene complexes with single molecule resolution using atomic force microscopy. *Eur. J. Oral. Sci*. **114**, 133–138.
16. Epstein V., Nudler E. 2003. Cooperation between RNA polymerase molecules in transcription elongation. *Science*. **300**, 801–805.
17. McAllister W. 1997. Transcription by T7 RNA polymerase. *Nucleic Acids Mol. Biol*. **11**, 1–-25.
18. Hansma H., Pietrasanta L., Golan R., Sitko J., Viani M., Paloczi G., Smith B., Hansma P. 2000. Recent highlights from atomic force microscopy. *J. Biomol. Struct. Dyn*. **18**, 271–275.
19. Limanskaya L.A., Limanskii A.P. 2006. Compaction of single supercoiled DNA molecules adsorbed onto amino mica. *Bioorg. Khim*. **32**, 494–510.