

Visualization of DNA 7 RNA polymerase complex by atomic force microscopy

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Complexes of bacteriophage T7 RNA polymerase (RNAP) with DNA template, containing promoter and terminator of T7 RNAP, at transcription elongation were visualized by atomic force microscopy (AFM). Images for both nonspecific (formed by T7 RNAP molecule with end fragments of DNA template) and specific (complexes of T7 RNAP with promoter and transcription termination area) complexes were obtained for pairs of single molecules. The influence of transcription parameters on the complex formation is discussed.

Keywords: *atomic force microscopy, AFM, transcription, T7 RNA polymerase, terminator, promoter, protein–DNA interaction.*

Introduction. Transcription is the process in the course of which RNA-polymerase (RNAP) forms a series of complexes at DNA-matrix scanning. In the beginning an unstable initiation complex is being formed, as a result of which, short products are synthesized before promoter is released. Later, the stable elongation complex is formed, consisting of RNAP, DNA-matrix and an increasing RNA-transcript [1]. At least three types of transition complexes were shown to form at transition from initiation to elongation complexes using the biochemical analysis and studying the kinetic aspect of interaction between RNAP and DNA matrix. The first transition complex consists of 3–5 nucleotides, the sec-

ond one of ~6–8 nucleotides, the third one of ~9–14 nucleotides [2, 3].

RNA-polymerases may be divided into two classes: oligomeric RNAPs (bacteria, eukaryotes) and monomeric RNAPs (some bacteriophages, mitochondria, chloroplasts). Though they have no structural homology or similar sequences, still basic transcriptional steps are identical for both RNAP classes [4].

The transcription is under polymerase control during elongation till the pause or termination. The stable elongation complex is dissociated in the process of transcription termination upon reaching specific position on the DNA-matrix. It is suggested that dissociation is not caused by termination immediately but first

inactivation of the elongation complex occurs [5]. Two types of signals for the pause or termination have been revealed for T7 RNA-polymerase, the most studied among monomeric RNAPs. Signals of class I contain U-rich element down the sequence, which forms GC-rich hairpin. The termination on class I sites is dependent on the possibility of secondary structure formation by the RNA molecule, which takes place even at the absence a non-matrix DNA chain. The other terminator of T7RNAP belongs to class II and consists of conservative sequence of 8 nucleotides. Generally, the pause or termination takes place on the site which is localized by 7–8 nucleotides down the aforementioned element. However, the details of both transcription initiation and termination are hard to reveal by means of traditional methods used for solutions, as, first of all, the initiation includes several rapid intermediate stages between binding to the promoter and elongation. Secondly, only the insignificant numbers of RNAP molecules in RNAP population which take the active part in transcription at every exact moment in time, are associated with the initiation [6].

The majority of biochemical investigations on the initiation of transcription is performed at experimental conditions which allow studying only fixed location of the enzyme, when PNAP is stopped at a known position of DNA matrix due to the absence of a complementary nucleotide. The attempts to synchronize the initiation of transcription for RNAP molecule population reached no success as the synchronization was abolished due to the stochastic nature of transition between the intermediate stages.

The other approach to study the transcription initiation, based on investigation on binding to the promoter, initiation, and elongation for a single out RNAP molecule in real time has been developed recently. This approach uses the DNA molecule, joined by both of its ends to two balls, located in optical traps. At the same time DNA molecule is located close to the surface where single immobilized T7 RNAP molecules are localized on. Guiding the optical trap by means of oscillation of the ball, to which one DNA molecule end is attached, the authors of [6] observed association and dissociation of the complex between T7 RNAP and promoter DNA, with the constant $K = 2.9 \text{ sec}^{-1}$, transition to elongation with $K = 0.36 \text{ sec}^{-1}$, synthesis at rate

of 43 nucleotides per second, and output length of RNA-transcript ~ 1200 nucleotides. Authors showed the transition from initiation to elongation lasting significantly longer comparing to the time of binary T7 RNAP-DNA-promoter complex existence.

In order to analyze RNAP-DNA complexes, formed at transcription, the method of atomic force microscopy (AFM) has been applied successfully [7]. High resolution AFM-visualization of protein-nucleic complexes allows direct study on biological structures at close to physiological conditions and without crystallization. Unlike traditional microscopic methods, which provide average information for the population of molecules, using AFM allows obtaining the data on structure and function of single molecules. Besides, a significant advantage of AFM, comparing to classic electron microscopy, is the absence of tinting or staining at preparing the sample. To study the topography of biomacromolecular surfaces using AFM the only requirement is for molecules to be adsorbed on a flat substrate. Therefore, a significant number of works is devoted to AFM-visualization of protein complexes with DNA, including RNA-polymerases [7, 8].

The current work presents the study on optimization of transcription conditions *in vitro* for subsequent AFM-visualization of the complex formed by monomeric T7 RNA-polymerase with linear DNA, containing promoter and the transcription termination area for T7 RNAP. Both non-specific (formed by T7 RNAP with terminal fragment of DNA-matrix) and high-specific complexes (first and foremost, with the promoter), which T7 RNAP forms with single molecules of DNA-matrix have been visualized.

Materials and Methods. *Transcription Amplicon* (length 1414 b.p.) with A1 promoter (1 of 17 known promoters, which T7 bacteriophage genome contains) and T7 RNA-polymerase transcription termination site was used as a transcription matrix (Fig.1). DNA template was obtained by the restriction by *ScaI* endonuclease (*New England Biolabs*, UK), supercoil DNA *pGEMEX* (length 3993 b.p.) (*Promega*, USA), with further amplification and purification of amplicon. Transcription reaction was conducted in accordance with the protocols given below using the transcription kits and T7 RNA-polymerases (*Promega*, USA), MegaScript T7 (*Ambion*, USA) and a kit from *New*

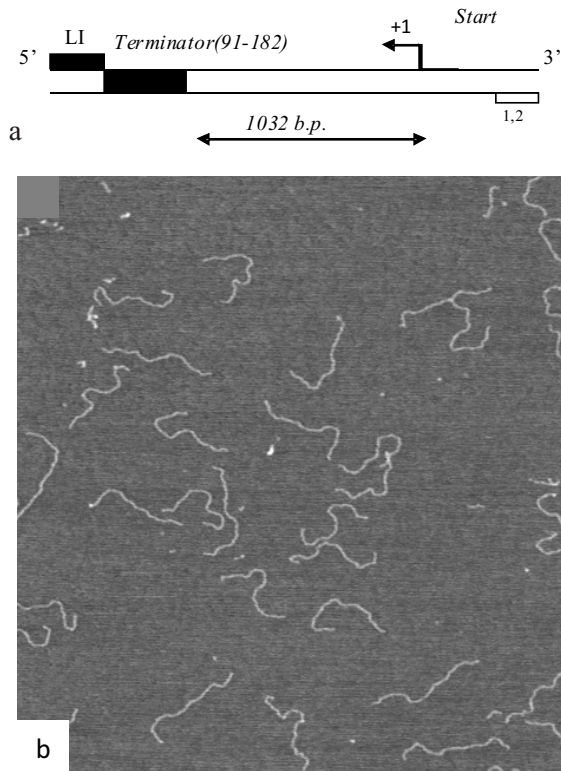


Fig.1 Schematic representation of linear DNA template used for transcription (a) and its AFM-image (b). Primers L1 and L2 (boxes) flank *pGEMEX* DNA fragment 1414 b.p. long containing the promoter and the terminator of T7 RNA-polymerase transcription. *pGEMEX* DNA was immobilized on freshly cleaved mica in HEPES buffer containing 2.5 mM of $MgCl_2$. Image resolution 2.23 $\mu m \times 2.23 \mu m$

England Biolabs, UK, at different temperature and time parameters.

Three transcription buffers were used: buffer *A* (*Promega*, USA), buffer *B* (*Ambion*, USA), and buffer *C* [8]. Buffer *A* contained 40 mM of Tris-HCl, pH 7.9, 6 mM $MgCl_2$, 10 mM NaCl, 10 mM dithiothreitol, 2 mM spermidine, 0.05 % tween 20, 40 units of RNazine, 20 units of RNA-polymerase in total volume of 20 ml. The transcription was initiated adding CTP, GTP, UTP, and ATP nucleotides to 200 μM .

Buffer *B* contained transcription reaction mixture, RNazine 40 units, 20 units of RNA-polymerase, 20 μM of CTP, GTP, UTP, and ATP in total volume of 20 ml. After 20-65 min of incubation at 31 C, the reaction was terminated by heating for 15 min at 70 C.

Buffer *C* contained 20 mM of Tris-HCl, pH 7.9, 5 mM $MgCl_2$, 50 mM KCl, 1 mM dithiothreitol, 40 units of RNazine, 20 units of RNA-polymerase in total volume of 20 ml. The transcription was initiated

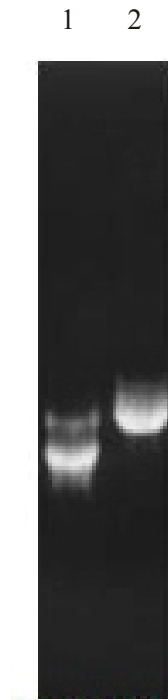


Fig.2 Analysis of RNA-transcripts after transcription with T7 RNA-polymerase on the template of *pGEMEX* linear DNA fragment 1414 b.p. long (line 1) at the denaturing conditions in 1.2% agarose gel with formaldehyde and control *pTRI-Xef* plasmid of 1890 b.p. (line 2). The expected length of transcripts was 1032–1122 nucleotides. Reaction was performed in buffer *A* (*Promega*) at 37°C, 1 h according to the manufac

adding CTP, GTP, UTP, and ATP to 100 μM . After 65 min of incubation at 31 C, the reaction was terminated by rapid cooling to 0 C.

To remove DNA template and depredated DNA, which may contaminate the RNA preparation, after transcription 1 ml of DNAase I, free of RNAase, (*Ambion*, USA) was added to the reaction mixture and incubated for 15 min at 37 C. DNAase was inactivated by incubating the reaction mixture at 70 C for 10 min. The transcription efficiency was controlled via electrophoresis in 1.2 % agarose gel, containing 1.8% of formaldehyde (Fig.2). The transcription was performed in a special room, set for working with RNA, using ultra-pure water with added DEPC to inhibit RNAases. Later, to perform the electrophoresis at denaturated conditions, the reaction mixture was added with equal volumes of buffer for sample applying; which contained 90% formamide, 0.01% xylencianol, 0.01% bromphenol blue, 10 mM EDTA, and 0.01%

SDS. To evaluate the sizes of RNA-transcripts, formed after the transcription on the DNA template *pGEMEX*, control *pTRI-Xef* DNA template (transcription kit *MegaScript T7* (*Ambion*, USA)) was used. Performing the transcription in accordance with manufacturer's conditions only full-size RNA-transcripts (length 1890 b.p.) were formed, and G 319A RNA-markers (*Promega*, USA) were used to evaluate their sizes.

Atomic Force Microscopy Atomic force microscope *Nanoscope IV MultiMode System* (*Veeco Instruments Inc.*, USA) with E-scanner was used in our work. AFM-images of DNA were recorded by the vibrating variant of AFM in the air, in "height"-mode using OMCL-AC160TS cantilevers (*Olympus Optical Co.*, Japan), resonance frequency 340-360 kHz and hardness constant 42 N/m. The images were obtained in 512 x 512 resolution, smoothed, and analyzed using *Nanoscope Software* (v. 5.12r3) (*Veeco Instruments Inc.*).

Preparation of DNA samples for PCR and T7 RNAP-DNA complexes for AFM To conduct polymerase chain reaction (PCR), L1 and L2 primers were constructed which bordered *pGEMEX* DNA fragment, containing promoter and termination site of T7 RNA-polymerase transcription. The L1 and L2 primers, the sequences of which with the corresponding positions on circular DNA *pGEMEX* are given below, were obtained from *Sigma*, Japan:

5'-cgc tta caa ttt cca ttc gcc att c-3' – direct primer L1 (3748–3772)

5'-ctg att ctg tgg ata acc gta tta ccg-3' – reverse primer L2 (1168–1142)

Hot-start PCR was conducted using GeneAmp 9700 amplifier (*Perkin Elmer*, USA) in the reaction mixture volume of 50 μ l at the following temperature and time parameters: initial incubation – 95 C, 2 min; denaturation – 95 C, 1 min; annealing – 71 C, 1 min; synthesis – 74 C, 1 min; number of cycles – 35. The temperature of annealing was determined theoretically using *Oligo* software.

Amplicons were visualized by separation of 15 μ l of PCR product using electrophoresis in 2% agarose gel with subsequent staining by ethidium bromide.

The following procedure was applied in order to purify the amplified DNA fragment. After electrophoresis, the band of gel, containing amplicon, was cut out

by long wave low intensity UV source (*BioRad*, USA). For further amplicon purification from nucleotides, primers, DNA-polymerase, and ethidium bromide, QIAquick PCR purification kit (*QIAGEN*, Japan) was used in accordance with the manufacturer's recommendations, as well as the extraction with phenol/chloroform with subsequent reprecipitation by ethanol.

PCR was performed using thermostable high accuracy DNA-polymerases of 2 types – *Pyrobest* DNA-polymerase (*TaKaRa Co.*, Japan) and *Invitrogen Platinum* DNA-polymerase (*Invitrogen*, Japan).

To apply T7 RNAP–DNA complexes on freshly cleaved mica, either 10 mM HEPES buffer, containing 2.5 mM $MgCl_2$ or the transcription buffer (as it also contained Mg^{2+} cations) was used. 10 μ l drop of mixture of complex was applied on mica line of 1 cm^2 , molar concentration ratio T7 RNAP and DNA in the range of 1–100 in TE-buffer (10 mM Tris-HCl, pH 7.9, 1 mM EDTA), after 2 min of exposition it was washed by RNAases free ultra-pure water, blown by nitrogen stream flow, and visualized immediately.

T7 RNAP-polymerase concentration in the output mixture was determined by spectral analysis using the control set of reagents for determining bovine serum albumin (BSA) concentration (BSA Protein Assay Reagent, *Pierce*, USA). The concentration of intermediate mixture of T7 RNA-polymerase was determined on the basis of calibration curve of optical density dependence on certain BSA concentration for 3 BSA concentration values, coming out from the measured value of absorption.

The concentration of T7 RNA-polymerase (*Promega*, USA) in stock solution was 6.6×10^{-6} M; the concentration of *pGEMEX* linear DNA, used as a template for transcription was 200–800 pM (amplicon) at AFM-visualization. To determine it, optical density and, thus, the concentration of purified PCR-product was measured, using spectrophotometry. The complexes were prepared by addition of T7 RNA-polymerases to equal volume of DNA template solution, obtained by the method of sequential diluting of the amplicon stock solution.

To visualize the complex of T7 RNA-polymerase–DNA, molar concentration ratio of polymerase and DNA was changed in the range of 1–100. The corresponding mixtures of T7 RNA-polymerase were ob-

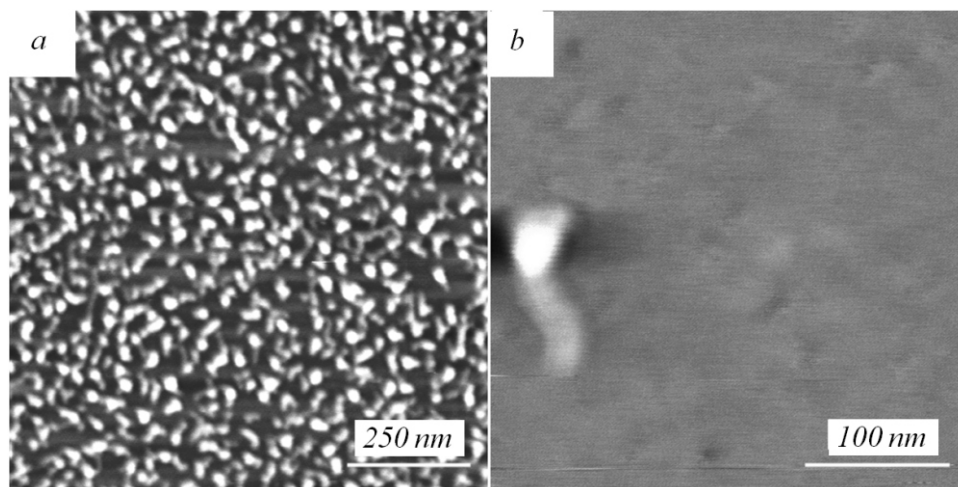


Fig.3 AFM-image of RNA-transcripts after transcription with T7 RNA-polymerase on the linear *pGEMEX* DNA of 1414 b.p. and treatment by DNAase I (expected length of transcripts was 1032–1122 nucleotides, image resolution a – 1 x 1 mkm; b – 315 x 315 nm)

tained by the method of sequential diluting of the stock solution.

Results and Discussion. To study the structure of T7 RNA-polymerase–DNA complexes using AFM, the immobilization of biomolecules on freshly cleaved mica adding Mg^{2+} ions was performed. AFM images of DNA template molecules, absorbed on mica (Fig.1, b) and being of non-extended shape and smoothed contour, the fragments of which were evenly immobilized on the substrate surface, were used as the control.

The transcription efficiency was controlled by the reaction with the control DNA-template – *pTRI-Xef* plasmid, which contained T7 RNAP transcription promoter, obtained from *MegaScript T7* kit (*Ambion*, USA). RNA-transcripts formed as a result of transcription on *pTRI-Xef* plasmid which were of 1890 b.p. length (determined after electrophoresis with RNA-markers (*Promega*, USA)) were used in turns as molecular mass markers.

The fact that the RNA-transcripts of specific length are synthesized as a result of transcription on *pGEMEX* DNA template was proved by the presence of corresponding bands on electrophoregram after denaturing electrophoresis of the transcription products with T7 RNA-polymerase (Fig.2).

The presence of two bands on electrophoregram testifies, to the author's mind, about the synthesis of RNA-transcripts of 1122 and 1032 b.p. (higher intensity band), which correspond to the transcription products with and without the transcription termination site. Regardless asynchronization of the reaction system, it is clear that after transcription only full-size

RNA-transcripts are formed and shorter transcripts, appeared after stop in the termination site. The transcription may be considered as scanning of DNA template by high accuracy RNA-polymerase at ~40 nucleotides per 1 sec [5]. Short RNA-transcripts of different length can also be formed, although their number is insignificant comparing to the number of full-size RNA-transcripts. Therefore, sensitivity of visualization of the transcription results using electrophoresis is insufficient for detecting these short RNA-transcripts. It is noteworthy that the situation with short RNA-transcripts is similar to the synthesis of during PCR not only amplicon of the expected length (amplification of which takes place in geometrical progression), but also of longer PCR-products, however, in a significantly lower concentration (synthesized in arithmetical progression), but they are not visualized on electrophoregram due to insufficient sensitivity of the system.

The current work is focused on the visualization of T7 RNAP–DNA complexes. However, RNA-transcripts, formed as a result of transcription, were also revealed (Fig.3). RNA-transcripts were similar to the condensed structures, as in other researches on AFM-visualization of RNA, because, to the author's mind, for visualization of extended non-condensed RNA molecules, the substrate surface features (mica) should be changed.

Having added T7 RNAP to the transcription mixture containing DNA-template in the process of transcription, elongational complexes, characterized by specific bends, typical for DNA-protein complexes, are

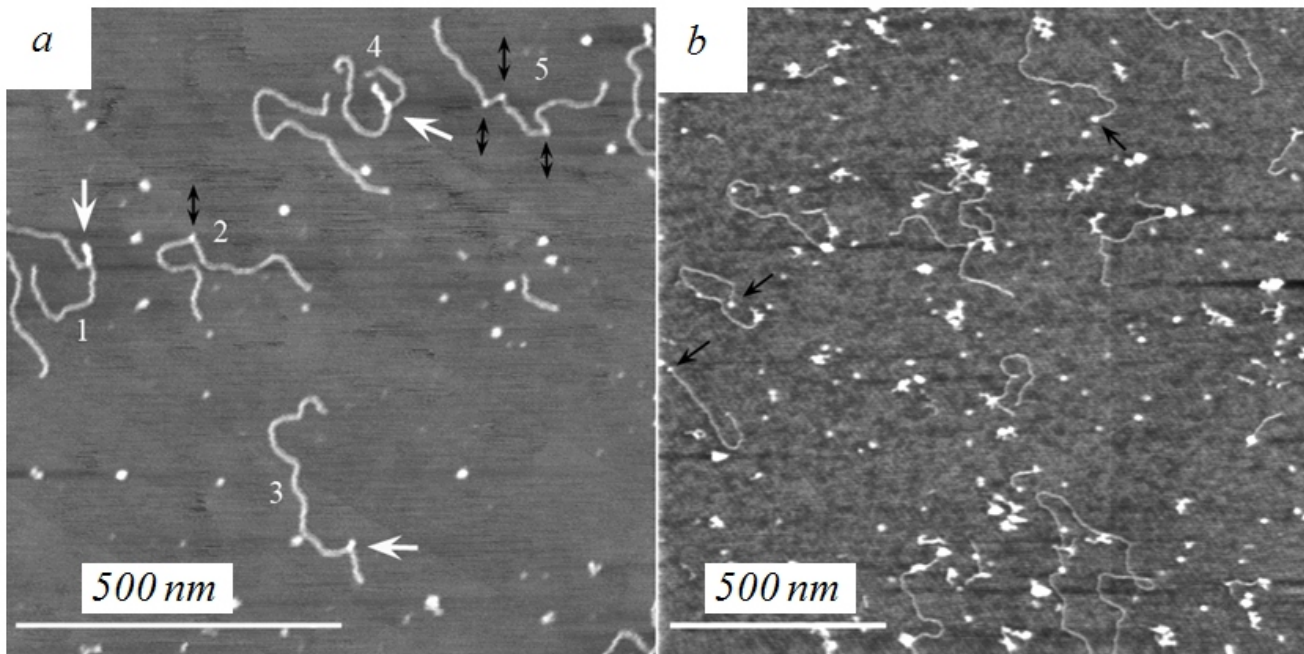


Fig.4 AFM-image of *pGEMEX* linear DNA fragment (1414 b.p.) used as transcription template and T7 RNA-polymerase molecules (two white points) after 60 min transcription in buffer A at room temperature (according to conditions [8]) (a) and after 65 min, at $T = 31^{\circ}\text{C}$ in buffer C for transcription (b); a – white arrows point at the coiled fragments of DNA-template which appeared as a result of complex formation with T7 RNAP molecules, black arrows point at T7 RNAP which formed the complexes inside the DNA-template; b – black arrows point at T7 RNAP molecules which formed the complex with the ends of the DNA template. Image resolution a – $1 \times 1 \text{ mkm}$, b – $1.48 \times 1.48 \text{ mkm}$. T7 RNA-polymerase to DNA ratio is 20.

formed [8, 9]. Fig.4 and Fig.5 show AFM-images of linear DNA *pGEMEX* after transcription with T7 RNA-polymerase at different temperature and time conditions and considerable excess of T7 RNA-polymerase molecules. DNA molecules in the complex with T7 RNAP are specific and of great interest for the presence of bends and formation of transcriptional loops after the transcription at room temperature (Fig.4, a, Fig.5). At the same time if T7 RNAP molecules are not present then bends and loops in *pGEMEX* linear DNA molecules are not seen at AFM images (Fig.4, b, [10]). In general, the formation of bends in the DNA molecule is determined by complex formation with proteins, on the one hand, while, on the other hand, the bends appear during visualization of DNA–protein complexes, absorbed on the mica surface. This effect may be briefly explained by the alteration of surface charge density of the DNA molecule upon the interaction with protein. Generally, the absorption of DNA molecules on the surface of evenly charged mica takes place due to electrostatic interaction of negatively charged DNA sites with positively charged surface groups of mica. As a result of such interaction of two evenly charged surfaces

(DNA and substrate), DNA molecules are regularly immobilized on the surface of substrate, which testifies to the existence of certain correlation (empirically established) between surface DNA properties and surface substrate properties.

The abovementioned facts allow concluding that DNA molecules are characterized by the conformation without bends only on mica surface, which has a certain value of surface charge density. At the change in the substrate surface properties, DNA molecules may be extended or compacted in the process of absorption on mica. The opposite situation is reasonable as well – local change in DNA surface charge (as it takes place in the process of interaction with protein) will result in immediate appearance of bends at immobilizing on the same substrate. As a result of electrostatic interaction of DNA with the protein, local surface density of the negative charge of DNA phosphate groups decreases, and, therefore, the repulsing forces of neighboring phosphate groups, which stabilize the DNA structure (along with stacking interaction of basic pairs), decrease as well [11, 12]. The result of this local change in the DNA surface properties in the sites of interaction with protein

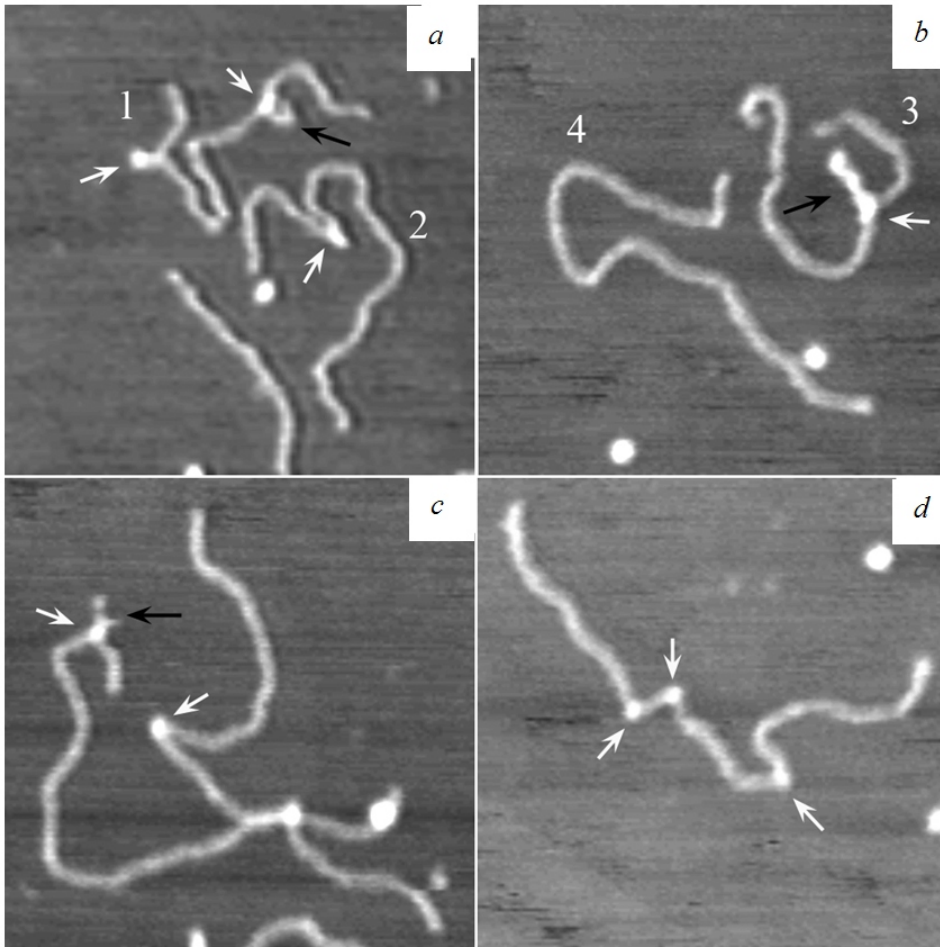


Fig.5 High resolution AFM-image of T7 RNA-polymerase and DNA-template complexes. T7 RNA-polymerase molecules are seen as spheres, $r \sim 8$ nm. White arrows point at the complexes formed by T7 RNA-polymerase and DNA, and the black ones point at RNA-transcripts. The transcription was performed in buffer A at room temperature for 60 min (similar to conditions in [8]), and then the reaction mixture was immediately used for AFM-visualization. Image resolutions: a – 348 x 348 nm, b – 306 x 306 nm, c – 298 x 298 nm, d – 299 x 299 nm. Contour length of molecules a – 445–450 nm, b – 454–457 nm

is an increase in conformational mobility of DNA at immobilization on mica, which causes the appearance of bends in the sites of DNA-protein interaction visualized on AFM images.

DNA-template for transcription was constructed to contain the promoter and T7 RNAP transcription termination site, which are localized asymmetrically at the ends of amplicon with the length of 1414 b.p. Thus, the transcription promoter is located between 1212–1231 b.p., *i.e.* 200 b.p. from 3'-end of the DNA template chain and the internal transcription terminator T7 RNAP is located between 91–182 b.p., *i.e.* 90 b.p. from 5'-end of the DNA template chain. Because there were no data in the available literature concerning the mentioned termination site, cloned in *pGEMEX* vector, the issue of detecting the termination signals for T7 RNAP arose on the basis of sequence analysis of the termination transcript. As a result of this investigation, a hairpin structure (with one unpaired nucleotide), 13

b.p. long with a loop 3 of n., was revealed and free energy of hairpin formation ($G = -16.4$ kcal/mol) was detected using GeneBee software [13].

AFM image (Fig.4, a) shows that DNA molecules No.1-4 formed the complexes with only one T7 RNAP molecule, while 3 polymerase molecules were bound to DNA No.5. At the same time DNA-T7 RNAP complexes for molecules No. 1, 3, 4 were qualitatively different from those No.2 and 5, the association of which is accompanied by the formation of specific bends. The fragments which may be interpreted as RNA-transcripts are shown with white arrows (note, that having done the thorough analysis of AFM images of similar size picture in [8], the authors did not reveal any DNA-RNAP complexes, containing increasing RNA-transcript).

The particular feature of AFM is the capability of measuring the contour length of DNA molecules with high resolution using software. Besides, one of the pri-

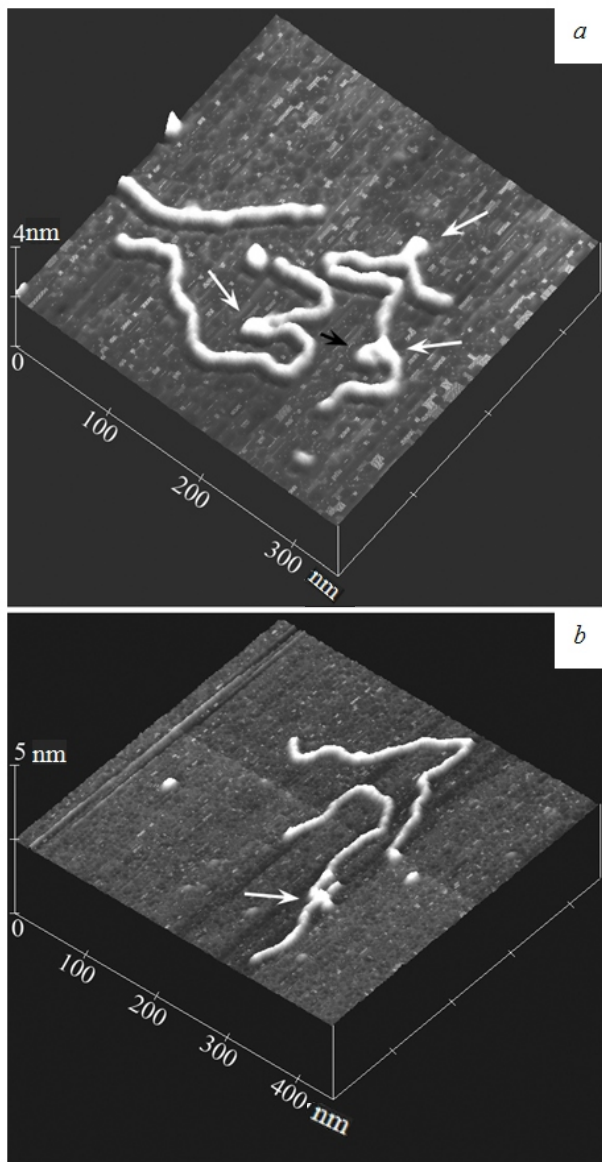


Fig. 6 High resolution AFM-image of T7 RNA-polymerase and DNA template for transcription at $T = 37^{\circ}\text{C}$, 4 min. Image resolution *a* – 280×280 nm, *b* – 280×280 nm. The arrow points at the complex formed by T7 RNA-polymerase and DNA. After transcription, 7 ml of buffer A were added to 7 ml of the reaction mixture and then it was applied on mica

mary applications of AFM was the measuring of DNA contour length [14].

In [10] the contour DNA-template length of 1414 b.p. after amplification was measured, which was used in this work to perform the transcription; the value equaled 435 ± 15 nm. The contour length of DNA molecules, which formed the complex with T7 RNAP were also in the range of this statistically reliable value 445–457 nm (Fig.5).

Let us take a closer look at the visualized complexes formed by T7 RNA-polymerase on both amplicon ends. Only one type of complex (Fig.6) has been discovered for transcription at $T = 37^{\circ}\text{C}$, 4 min – T7 RNAP molecule is bound to one of DNA-template ends. The height of this complex (Fig.6, *b*, arrow), measured from AFM image by means of cross and longitudinal section (results are not presented), equaled ~ 0.5 nm which corresponds to the value of height of a single T7 RNAP molecule. At the same time, two types of complexes (T7 RNAP complexes with promoter or terminator) localized on both ends of DNA template (Fig.7) were visualized after transcription at $T = 31^{\circ}\text{C}$ for 65 min. The height of the other T7 RNAP-DNA complex indicated by a black arrow (Fig.7, *a*, *b*) was 0.93–1 nm.

It is noteworthy that both specific and non-specific complexes are formed at the interaction of DNA molecule and T7 RNAP. Specific binding (interaction with promoter) is relatively insensitive towards changing in ionic strength of mixture, but it depends on conformation of the DNA fragment. Non-specific complexes of DNA-RNAP complexes are formed due to electrostatic interaction of positively charged polymerase residues with negatively charged phosphate groups of DNA. Non-specific binding is highly sensitive toward changing in ionic strength of the reaction mixture, however, it is insensitive to the degree of DNA supercoiling. Having analyzed the features of non-specific and specific DNA-RNAP complexes, the authors of [15] made the conclusion that the basic constituents of regulation mechanism of T7 bacteriophage transcription are the efficiency and the rate of RNAP open complex formation with promoter, but not differential promoter binding.

The structure of complex, formed by T7 RNAP polymerase at transcription, has been studied thoroughly in the previous investigations. Thus, in [16] X-ray analysis was used to present detailed crystalline structure of the elongation complex of T7 RNA-polymerase with resolution of 2.9 Å. RNA-DNA hybrid (8 b.p.) was shown to be built in the active center of the enzyme, resulting in significant rearrangement of the amino end domain. Such rearrangement includes alternative folding of about 130 residues and significant reorientation (with 130° turn) of core subdomain, resulting in a structure formation which provides

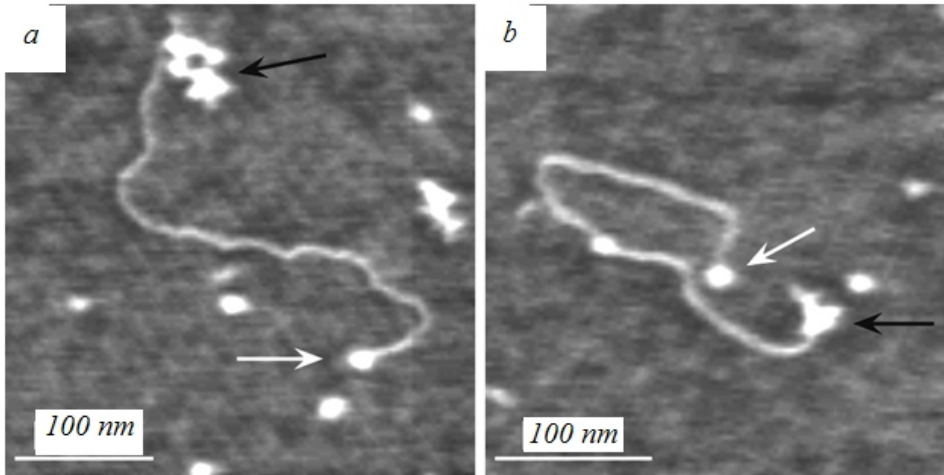


Fig.7 High resolution AFM-images of DNA-T7-polymerase complexes formed after 65 min, 31°C transcription in buffer C. T7 RNA-polymerase to DNA ratio was 10. White arrows point at T7 RNA-polymerase with the promoter, and the black ones point at the complex in the transcription termination site. Reaction was terminated by rapid cooling down to 0°C. To perform AFM-visualization the reaction mixture was 10 times dissolved with HEPES buffer. Image resolution: *a* – 338 x 338 nm; *b* – 297 x 297 nm.

proper operation of stable elongation complex. Though much data are published on T7 RNAP structure in elongation and initiation complexes, the data on these complexes obtained with biochemical method do not match the crystallographic information completely. Using kinetic analysis of the transcription initiation and elongation, the stage of initiation was determined to last 4 sec for single T7 RNAP molecules [4] and RNA-transcript of 1200 b.p. synthesis lasts approximately 30 sec. Therefore, in our case performing the transcription for 4 min is enough for the synthesis of RNA-transcripts on DNA-template of 1414 b.p. used in our work. However, at 4 min transcription (in buffer A) only the complexes which are specific for binding to one DNA-template end, similar to the variant in Fig. 6, *b*, were visualized. At the same time the increase to 65 min in transcription time allowed detecting T7 RNAP complexes with both terminal fragments of the DNA-template (Fig. 7).

The authors believe that the complexes indicated by black arrows in Fig.7 correspond to the complex formed by T7 RNAP with the DNA-matrix in the transcription termination site. It is possible that after elongation, several T7 RNAP molecules with corresponding RNAP-transcripts “stop” at the terminator one by one. It is supported by rather a significant size of the complex, as well as the presence of T7 RNAP molecule inside the DNA-template at the complexes on both terminal sites of the DNA template.

Generally, to stabilize the complex at the transcription termination, the reaction is performed at the average ionic strength (*I*) 75–80 mM Na⁺ and NTP concentration 80–200 mM. The transcription in our work was performed at *I* = 80 mM Na⁺ (buffers A and C, the manufacturer of buffer B does not expose the reaction mixture content). The RNAP termination mechanism of T7 phage is less studied and comprehended, comparing to the cell termination mechanism of *Escherichia coli* RNAP, as the majority of elongation complexes of T7 RNAP are non-stable and are easily dissociated even at the condition of RNAP stop outside the termination site. It is also supposed that in case of ionic strength decrease, the termination complex at reaching the termination site is dissociated fast (5-15 sec). It is considered to be the common feature of the internal terminators [17]. Besides, this complex is dissociated fast even at not high ionic strength values, meanwhile in *E. coli* RNAP the majority of elongational complexes, including the complexes which stop at the positions of transcription termination are stable and can be visualized [8].

To our mind, the visualization of a great number of T7 RNAP complexes with terminal fragments of the DNA-template may be explained by several reasons. Firstly, the promoter and the termination site are localized on the ends of the DNA-template. As the binding constant for T7 RNAP molecule with highly specific promoter site exceeds that of non-specific binding

(with any site inside the DNA template) [15], then at the dissociation conditions of T7 RNAP-DNA complexes the promoter-T7 RNAP complex is the most stable among the ones, formed in the processes of transcription initiation and termination. Secondly, the dissociation time of T7 RNAP-promoter complex is much longer than the time of transition to the elongation complex; therefore, there is a higher possibility of complex visualization with the promoter at different equal conditions. Thirdly, pause or complete stop of the enzyme takes place in the termination site which statistically increases the possibility of complex visualization with the terminal DNA-template fragment.

The key result of current investigation is the direct visualization of the complexes formed by T7 RNAP with terminal fragments of DNA-template as well as with promoter and transcription termination site. The data obtained allow supposing that the process of scanning by RNA-polymerase starts from the end of DNA template nearby the promoter. Visualization of several T7 RNAP molecules in the complex with DNA-template confirms the results of [18], which presents the possibility of association of several RNAP molecules in the process of transcription elongation.

The study on the complexes formed by RNAP during transcription may be of great importance for understanding the resistance of RNAP toward antibacterial preparations which is often discussed in the literature. Besides, from the standpoint of future AFM-investigations, it is worth studying the elongation complexes of clinical isolators of *Mycobacterium tuberculosis*, RNAP of which is more than 1000 times more sensitive to anti-tuberculosis antibiotic rifampicin comparing to RNAP of *E. coli* [19].

The results obtained give grounds for hoping that AFM will remain a powerful and effective method of visualization of macromolecular complexes and investigation of intermolecular interactions in DNA-protein complexes, especially for not dried samples, but in buffer directly at the physiological conditions, providing advanced understanding of the complicated transcription regulation mechanisms.

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А. П. Лиманский

Визуализация комплекса ДНК с T7 РНК-полимеразой с помощью атомно-силовой микроскопии

Резюме

С использованием атомно-силовой микроскопии (АСМ) визуализированы комплексы РНК-полимеразы (РНКП) бактериофага T7 с ДНК-матрицей (содержащей промотор и терминатор транскрипции T7 РНКП) при проведении транскрипции. На уровне пар единичных молекул получены изображения как неспецифических (образованных молекулой T7 РНКП с концевыми фрагментами ДНК-матрицы), так и специфических (сформированных T7 РНКП с промотором и областью терминации транскрипции) комплексов. Обсуждается влияние параметров транскрипции на комплексобразование.

Ключевые слова: атомно-силовая микроскопия, АСМ, транскрипция, T7 РНК-полимераза, терминатор, промотор, взаимодействие белок-ДНК.

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