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Molecular Mechanisms of Transcription through Chromatin by RNA Polymerase III: Part 2

V. M. Studitskii, I. V. Orlovskii, O. V. Chertkov, N. S. Efimova, M. A. Loginova, and O. I. Kulaeva

Laboratory of Transcription Regulation and Replication, Department of Bioengineering, Biological Faculty, Moscow State University

e-mail: studitvm@umdnj.edu

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Abstract—Nucleosome forms a so-called nucleosome barrier, which is a serious difficulty for a transcriptional apparatus. We considered studies of formation and nucleosome barrier closing by some types of RNA polymerases. We showed that different polymerases use similar mechanisms for chromatin transcription. We present data testifying to the high similarity between transcriptional and ATP-dependent chromatin remodeling. We propose a nucleosome-mediated model of transcription.

Keywords: chromatin, transcription, nucleosome, nucleosome barrier, chromatin remodeling, elongation, RNA polymerase III.

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In the first part of the review, chromatin structure and the general principle of chromatin-organized DNA transcription are discussed. In this part, we will also discuss additional aspects of chromatin transcription.

A nucleosome that forms a nucleosome barrier is a serious block for the elongation complex. In the case of bacteriophage SP6 RNA polymerase transcription, the nucleosome barrier was studied using short mononucleosome matrixes that allow RNA polymerase blocking in certain positions in the nucleosome [1]. Thus, polymerase was blocked in a position where a pause occurred (42 b.p. from nucleosome beginning), as well as in a position before the nucleosome barrier (23 b.p.). Structure of intermediates forming at different positions in the nucleosome was analyzed by electron cryomicroscopy (ECM). This data was compared with biochemical analysis data obtained from the same samples. During transcription through the nucleosome, three types of intermediates are formed (Fig. 1): (a–f) particles with one DNA tail, in some cases, (a, e, f) complex polymerase-RNA is situated near the octamer, in some cases, free DNA is observed between the complex and the octamer (b–d); and (g–j) particles with two DNA tails. Expected polymerase-RNA complexes are electron dense regions depicted by arrows. (k, l) Particles with one DNA tail where the DNA region proximal to the promoter is connected with a nucleosome; such conformation can lead to the formation of an intermediate with a loop. The scale is 10 nm. At the top, there is a three-dimensional model of the complex *a* shown at two angles. The nucleosome is represented as a disk; the transcript is shown as a black cylinder. Data, including identification of polymerase-RNA complexes, is interpreted based on the comparison between original and processed microphotographs of stereopairs. Such processing allows DNA visualization but complicates transcript determination. Nucleosomes were formed on a matrix with length 227 b.p.; they were transcribed by bacteriophage SP6 RNA polymerase. In Fig. 2, there are three classes of complexes which are formed during nucleosome transcription: a is particles with one DNA tail when polymerase-RNA complexes (arrows) are situated similar to histone octamer; b is with free DNA between octamer and polymerase; c is particles with two DNA tails. Complexes *a* and *c* are closed intermediates and are observed only when RNA-polymerase comes to the nucleosome barrier. Scale is 10 nm. Three-dimensional models of complex *a* are presented. The nucleosome is represented as a disk; the transcript is as a black line [1].

Complexes *a* and *c* were observed only when polymerase came to the barrier. Only two mentioned closed intermediates can be observed at polymerase spontaneous stops at different positions of the nucleosome. Therefore, presence of closed intermediates correlates with nucleosome-specific pauses. It can be suspected that such intermediates are responsible for pauses; for prolongation of transcription processes, they need to be transformed into an open form (Fig. 2). It should be noted that, according to electron microscopy data, only one supercoiled DNA turn associated with the octamer remains on the nucleo-

some in all transcription intermediates. This permits us to suppose that the second DNA turn is sterically expelled by transcribing polymerase that is situated at the neighboring turn of DNA supercoil.

Surprisingly, there is no transcription pause at nucleosome borders [1]. It can be expected that histones bound to DNA can be seen by polymerase and that intermediates forming during nucleosome transfer participate in the formation of transcription nucleosome barrier. Perhaps stronger DNA-histone interactions typical for central region of nucleosome DNA stop RNA polymerase only after transcription of 20–30 b.p. nucleosome DNA. Studies of nucleosome transcription by RNA polymerase 2 (see below) show that both mechanisms participate in the formation of nucleosome barrier [1].

Based on the presented data, we proposed the following mechanism for transcription at nucleosome (Fig. 3: 1—RNA polymerase (RNAP) rapidly transcribe first 25 b.p. of nucleosome DNA; (2) DNA partly dissociate to octamer. 3—DNA behind RNAP binds to free surface of histone octamer, thus forming internucleosome loop). (4, 5) Further, RNAP moving leads to loop break and formation of intermediates with RNAP that is situated just beside the nucleosome. Here RNAP stops. DNA then seems to dissociate from octamer surface and configuration 2 is restored. This cycle can be repeated several times. When polymerase passes approximately 60 b.p. of nucleosome DNA, upstream DNA dissociates from octamer surface forming conformation 6; octamer transfer in finished (7) [1]. After transcription initiation (intermediate 1) and entry into nucleosome, RNA polymerase transcribes 20–25 b.p. of nucleosome DNA without stops and sterically displaces at least 20 b.p. from the surface of histone octamer before the elongation complex (intermediate 2). This way polymerase clears its way; this can explain why histone octamer is almost completely clear for transcription. When polymerase transcribed more than 25 b.p. of nucleosome DNA, internucleosome loop (intermediate 3) is formed as a result of interaction between free octamer surface and downstream DNA. The size of this loop depends on the length of the downstream DNA region available for interaction and varies within 10–900 b.p. Internucleosome loop can be opened from any part of polymerase: at the early stages of transcription, release of proximal end of matrix is the most favorable. When DNA turns around the histone octamer, firstly, intermediate 4 with one free DNA tail is formed. While polymerase displaces another DNA end, intermediate 5 with two free DNA tails is formed. Up to 50 DNA b.p. in it are displaced with the elongation complex. Notably, the displaced region is situated approximately 80 b.p. before polymerase. As a result of such distant displacement, the elongation complex is not surrounded by a large number of DNA-histone interactions during transcription through the nucleosome. This can facilitate polymerase escaping pause state.

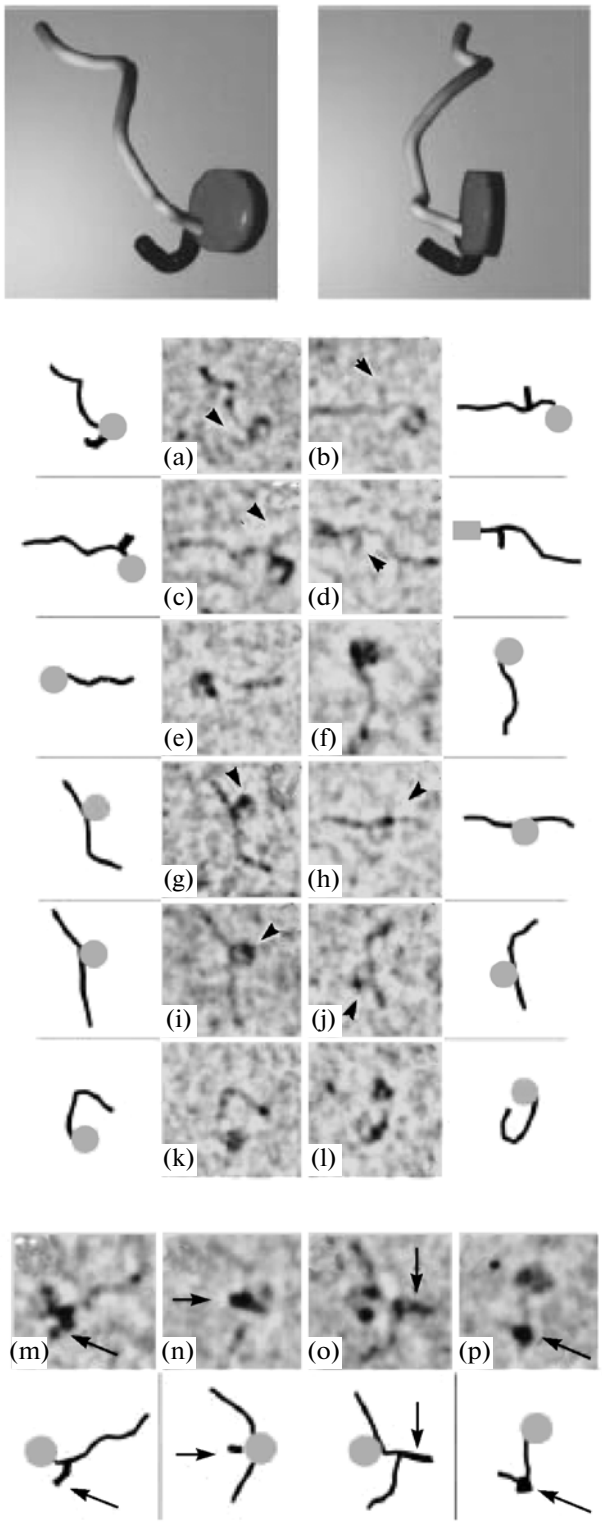


Fig. 1. Data of cryoelectronic microscopy for (a–l) stopped and (m–p) transcribing transcription complexes [1].

Elongation stops until further DNA displace from the surface of histone octamer will allow to return to intermediate 2. This permits approximately 10 b.p. move of

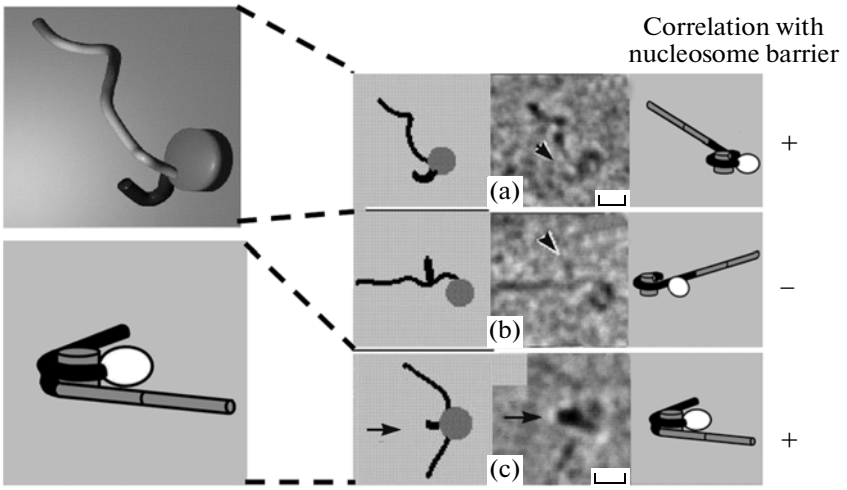
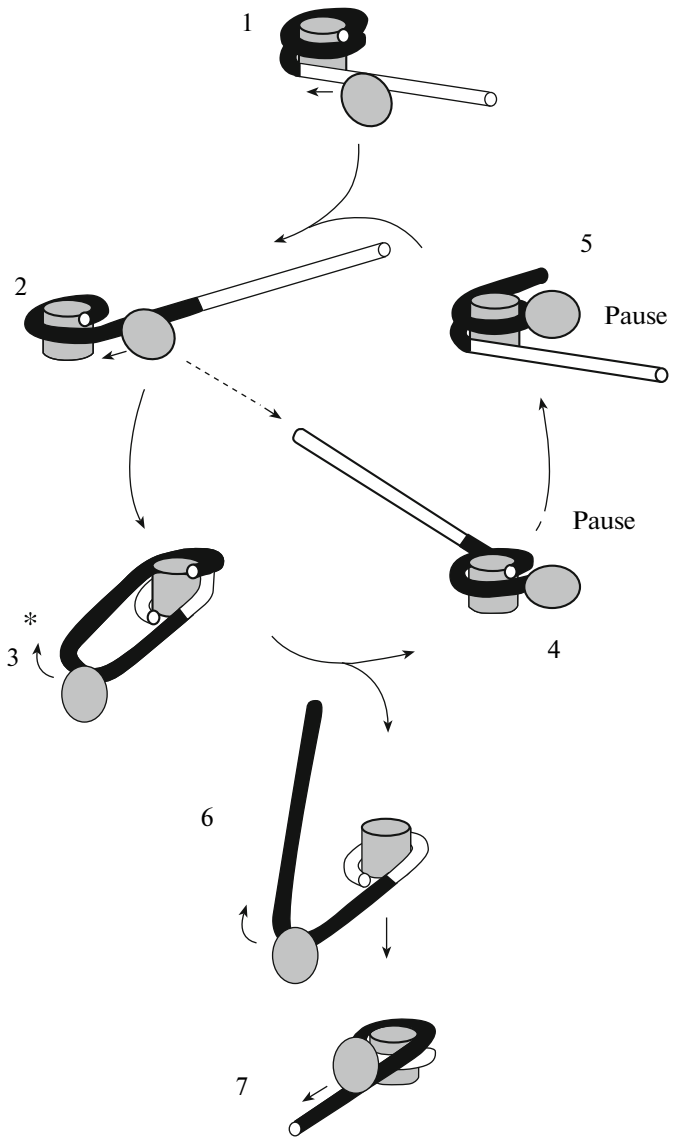


Fig. 2. Structure of nucleosome barrier for transcription: identification of intermediates forming during nucleosome-specific transcription pause using electron cryomicroscopy (ECM) [1].



RNA polymerase along the matrix. Alternate formation of opened and closed transcription complexes occurs several times as RNA polymerase moves inside nucleosome core for up to 60 b.p. Finally, DNA-histone interactions behind polymerase become stronger than interactions before the elongation complex and the nucleosome is restored in new region behind transcribing polymerase (intermediate 6). Upon reaching this stage, further transcription runs without delay through free DNA formed in front of polymerase (intermediate 7).

Can the mechanism of nucleosome transcription by small (approximately 100 kDa) one-subunit bacteriophage SP6 RNA polymerase be similar to the mechanism used by predeterminedly larger (up to 600 kDa) polysubunit eukaryotic RNA polymerases? An attempt to answer this question was made in the research where transcription of short mononucleosome matrix by bacteriophage SP6 RNA polymerase and east RNA polymerase 3 was studied. This allowed direct comparing of prokaryotic and eukaryotic RNA polymerases transcription at similar matrixes in similar conditions (Fig. 4: at the top: analysis of nucleosome-specific pauses during transcription of similar 227 b.p. by east RNA polymerase 3 and bacteriophage SP6 RNA polymerase. Analysis of labeled transcripts was performed in denaturing PAAG. Nucleosomes and DNA were transcribed for different time (2, 4, 8, 30, 180 s and 4, 10, 5, 60, 180, 600 s for RNA polymerase 3 and bacteriophage SP6 RNA polymerase, respectively). Pauses were observed for transcription of common regions (25–60 b.p. from nucleosome begin)

Fig. 3. Mechanism of nucleosomes transcription by bacteriophage SP6 RNA polymerase (and RNAP3, see Fig. 5) [1].

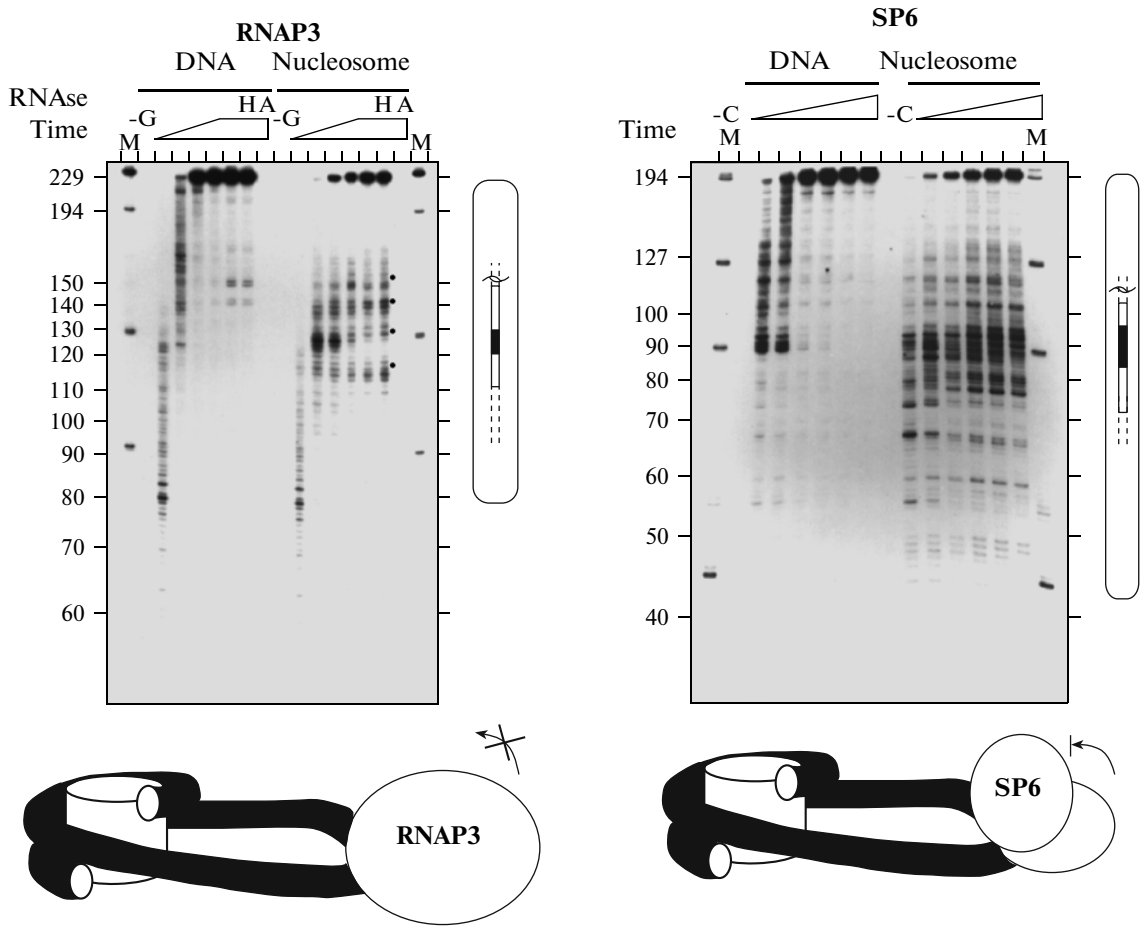


Fig. 4. Transcription through nucleosome by prokaryotic and eukaryotic RNA polymerases occurs according to similar mechanisms [2].

for both polymerases. But pauses were only observed for RNA polymerase 3 at intervals of 10 b.p. (depicted by spots). The position of nucleosome is depicted on the right; regions of strong nucleosome-specific pauses are depicted by black blocks, less strong are depicted by white blocks, and weak ones are depicted by dash lines. The position of nucleosome dyadic axis is indicated. M is RNA markers. At the bottom on figures: probable structures of nucleosomes transfer intermediates [2]).

Both distribution of nucleosome-specific pauses during transcription and distance of histone direct transfer were equal in two cases (Fig. 4). This data shows that rather different polymerases use similar mechanisms for transcription through nucleosome. As SP6 bacteriophage RNA polymerase does not transcribe nucleosomes in vivo, this testifies to the fact that transcription mechanism through nucleosome reflects certain differences in fundamental properties of elongation complexes and nucleosome structure [2].

Can the loop mechanism similar to transfer transcription mechanism work in the case of ATP-dependent chromatin remodeling? The following data indicates the high similarity in mechanisms of transcription and ATP-dependent chromatin remodeling [3, 4]. Firstly, similarity in many effects of ATP-dependent remodeling and RNA polymerases enzymes functioning, e.g., nucleosomes translocations to short distances and part DNA displacement from octamer surface and octamer transfer in trans (between different DNA molecules). These effects seem to be connected with local DNA unwinding from histone octamer during transcriptional and ATP-dependent remodeling. Secondly, histone octamer is intact after translocation. Thirdly, both types of chromatin remodeling are connected with nucleoside triphosphates (NTPs) hydrolysis. Finally, at least some ATP-dependent remodeling complexes (remodelers) can move along DNA. All mentioned complexes have marked helicase-like motives (but they have no helicase activity) and are DNA translocases that can move directionally along DNA possibly wheeling around double helix. Therefore, ATP-dependent remodelers have all properties typical for transcribing RNA polymerase. It seems that they determine nucleosome fate during the appropriate process. Perhaps ATP-depen-

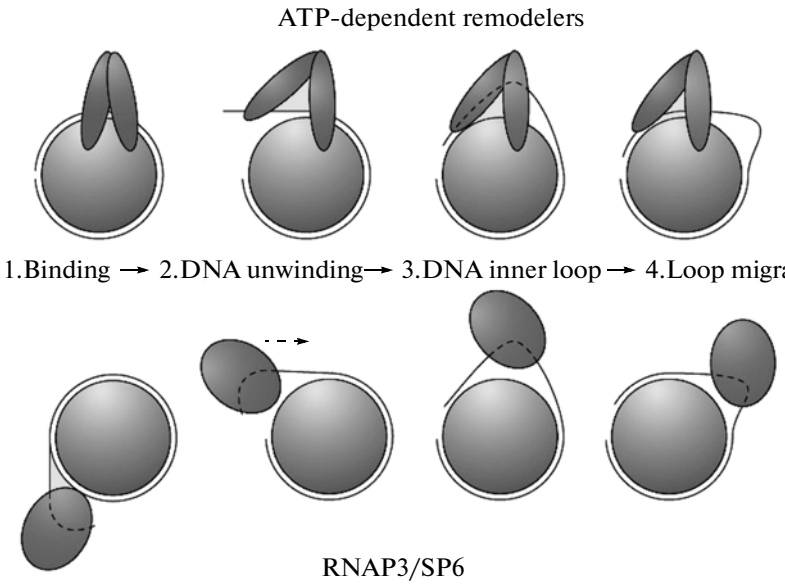


Fig. 5. Loop mechanisms of chromatin remodeling by certain ATP-dependent remodelers (above), east RNAP3, and bacteriophage SP6 RNA polymerase.

dent remodelers and RNA polymerases use similar mechanisms during transcription (Fig. 5: elongating RNA polymerase gives ~90° turn in DNA. (1) Binding: remodeling complex moves near nucleosome. (2) DNA is locally unwinding from octamer surface. (3) Remodelers give the turn into DNA facilitating formation of internucleosome DNA loop. It is proposed that ATP-dependent remodelers have both DNA-binding and octamer-binding sites and that ATP hydrolysis leads to change in protein complex conformation, thus easing turn possibility in DNA. (4) DNA turn moves inside nucleosome DNA spontaneously (ATP-dependent remodelers) or together with transcribing enzyme). These mechanisms also can be used in other mechanically similar processes such as DNA replication. Indeed, histone octamer is transferred without histones displacement in solution during in vitro replication of virus SV40 microchromosomes. In addition, DNA is effectively replicated without octamer dissociation from DNA.

Therefore, the use of short (220–250 b.p.) mononucleosome matrixes is useful for study of some key properties of transcribed chromatin, e.g., nucleosome maintenance during transcription. Using short matrixes allowed us to find a range of surprising characteristics for transcription mechanism of DNA packed in chromatin. The most notable are acrobatic abilities of histone octamer, which possibly facilitate RNA polymerase passing through nucleosomes even without short time octamer dissociation and its release to solution. Affinity of histone octamer to DNA is very high, while it is almost clear for many transcribing polymerases (e.g., during transcription of mononucleosome matrixes by bacteriophages SP6 RNA polymerase). Nevertheless, single nucleosome situated both on short (200–250 b.p.) matrix and on long polynucleosome matrixes is a serious difficulty for elongation complex. Intermediates of histone octamer transfer seem to be the main barrier slowing transcription as formation of such intermediates correlate with formation of nucleosome barrier for transcription.

Is the above described mechanism specific for RNAP3 used for transcription in vivo? Results of nucleosome transfer model application to polynucleosome regions of transcribed genes are presented on Fig. 6: each complex of transcribing RNAP results in each nucleosome transfer to the direction of promoter by reeling mechanism (see Fig. 4). This leads to reduction in nucleosome number in the part of gene distal from the promoter. This process can be compensated by nucleosome reassembling. Each octamer displaced to promoter occupied by transcription factors is displaced and releases to solution or is directly transferred into another region (e.g., to the part of gene distal to promoter) [5]. Since nucleosomes move in the direction opposite to the direction in which RNA polymerase is moving during transcription, nucleosomes are moving toward promoter. The number of nucleosome on the distal gene part decreases. This can be inhibited by simultaneous nucleosomes assembling, as well as by direct transfer of certain histone octamers to gene regions free from nucleosomes. Histone octamers transferred into a promoter direction will be displaced into solution since transcription initiation factors and ATP-dependent remodelers situated on active promoters prevent nucleosomes assembling. Such shuttle model supposes dynamic organization of nucleosomes on genes transcribed by RNAP3

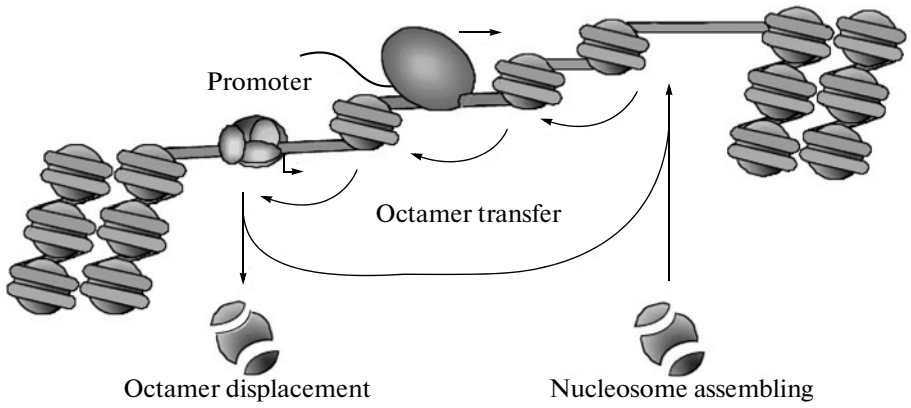


Fig. 6. Hypothetic shuttle model of nucleosome organization in active gene transcribed by RNAP3 [5].

and stepwise transfer of a wide range of nucleosomes to the promoter direction during each transcription. Indeed, recent genomic studies of chromatin transcribed by RNAP3 showed that appropriate genomic regions are being actively remodeled during transcription and perhaps are access points for factors participating in transcription genome regulation [6]. Therefore, experimental data together with the above presented arguments allow us to consider that the described nucleosome transcription mechanism (Figs. 3, 6) appears to be used in vivo, at least in the case of genes transcribed by RNAP3. It should to be noted that mechanisms of chromatin transcription by RNAP2 and RNAP3 are significantly different [7, 8].

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