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REVIEWS

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Molecular Mechanisms of Transcription through a Nucleosome by RNA Polymerase II

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Abstract—The RNA polymerase II (Pol II)-type mechanism is conserved from yeast to human. After transcription initiation, Pol II usually pauses upstream of or in a nucleosome within the early transcribed region of a gene. Then Pol II overcomes the initial nucleosomal barrier and efficiently proceeds through chromatin. At a low to moderate transcription rate, Pol II progression is only characterized by displacement or exchange of H2A/H2B dimers, and the resulting hexasomes (subnucleosomes lacking one of the H2A/H2B dimers) survive owing to the formation of small intranucleosomal DNA loops. The nucleosome structure is restored before the next Pol II complex starts transcription at a moderate transcription rate. The mechanism most likely helps to preserve the histone code during transcription. As the transcription rate increases, the distance between transcribing Pol II complexes becomes shorter, and trailing Pol II complexes may encounter the hexasome formed in the previous transcription round before the H2A/H2B dimer rebinds to it to restore a complete nucleosome. An unstable intermediate with fewer DNA–histone contacts forms in this case, resulting in the eviction or exchange of all core histones during transcription. Various protein factors and histone chaperones are involved in chromatin transcription by Pol II in vivo.

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Keywords: chromatin, transcription, nucleosome, nucleosomal barrier, RNA polymerase II, FACT

INTRODUCTION

RNA polymerase II (Pol II), which is responsible for gene transcription, acts as a heteromultimeric complex of 12 different subunits with a total molecular mass of 0.5 MDa [1]. During transcription, Pol II turns around the DNA helix every 10 bp, progressing through highly condensed chromatin [2]. The nucleosomal packaging in chromatin substantially hinders both the access of enzymes and transcription factors to DNA and the progress of transcribing Pol II. Thus, nucleosomes regulate the DNA accessibility, preventing or facilitating the binding of transcription factors and RNA polymerases [3–8]. After transcription initiation, Pol II pauses after transcribing the first 50–100 bp on many eukaryotic genes [9–11], often because the enzyme encounters the next, (+1) nucleosome, which is at the start of the transcribed gene region [12, 13]. Once the (+1) nucleosomal barrier is overcome, transcription of the downstream extended DNA region (up to more than several hundreds of kilobases) packaged in nucleosomes proceeds at a high rate, 3–4 kb/min [14]. A similar transcription rate is observed in vitro on histone-free DNA [15, 16], indicating that the (+1) nucleosome is one of the key factors in regulating transcription at the elongation stage.

Further transcription of a gene by polymerase is associated with various changes in nucleosomal structure with temporary disruption of DNA–histone interactions. At a high transcription rate, all of the core histones (including H3/H4) are partly lost [6, 17–20] or exchanged [21–26] in transcribed gene regions. Nucleosome loss is not observed at a moderate transcription rate, which is characteristic of the majority of transcribed genes [17–19, 27, 28]. Rapid transcription-dependent exchange of histones H2A/H2B, but not H3/H4, is observed for these genes [23–25]. In addition, the histone octamer is temporarily unfolded as a result of transcription. An intact (nontranscribed) nucleosome is known to lack reactive SH groups, while SH groups of histones H3

Abbreviations: ZLS, zero loop-stabilizing, negatively charged regions of RNA polymerases; FACT, Facilitates Chromatin Transcription, a transcription factor; PolII, RNA polymerase II; PolIII, RNA polymerase III; EC, elongation complex; AC, active center; PAGE, polyacrylamide gel electrophoresis; HA, high affinity.

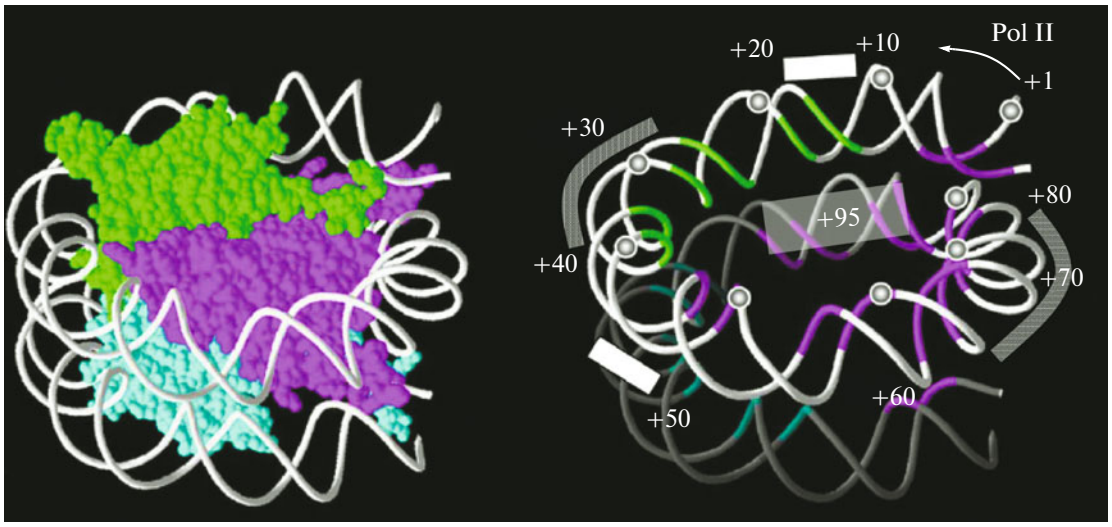

Fig. 1. Positions of the nucleosome-specific barriers (white lines) to Pol II transcription in the core nucleosome structure. Gray lines show the DNA–histone interactions that affect the barrier strength. The structure of the core nucleosome (on the left) and the nucleosomal DNA folding (on the right) are shown. The H3/H4 tetramer is purple; the H2A/H2B dimers are green and blue. The DNA–histone interaction sites are colored accordingly (on the right). The transcription direction is shown with an arrow. The interactions in regions (+35), (+70), and (+95) determine the strength of the (+15), (+45), or (+45) barriers, respectively.

become accessible for various probes in transcribed genes [39–31]. The accessibility of SH groups correlates with transcription [29, 30, 32]. Alterations of the nucleosome structure are temporary during transcription in vivo, and preservation of the chromatin structure is absolutely essential for the normal cell function [33, 34]. Irreversible loss of histones facilitates the formation of a chromatin structure accessible for various DNA-damaging agents [35, 36] and leads to loss of cell viability or early cell aging [34].

NUCLEOSOMAL BARRIER

Studies of transcription through nucleosomes in vitro showed that Pol II pauses at certain positions on nucleosomal DNA [37]. Two preferential Pol II pausing sites were identified, where the active center (AC) of the enzyme is approximately 15 or 45 bp away from the nucleosomal DNA boundary proximal to the promoter (hereafter referred to as (+15) and (+45) sites) (Fig. 1). The Pol II progress through the (+15) and (+45) sites is similarly retarded in transcription of nucleosomes formed on randomized DNA sequences. The sites are presumably adjacent to sites of DNA–histone interactions of higher activity, which only partly depends on the DNA sequence. The (+45) barrier is stronger and determines the total rate of Pol II progress through nucleosomes on the majority of nucleosomal templates [37].

To map the histone regions that are involved in these interactions and determine the strength of the nucleosomal barrier to Pol II, transcription was studied with nucleosomes containing various mutant histone variants. Four Sin mutations decreasing histone H3 or H4 affinity for nucleosomal DNA were selected for experiments [38]. All of the mutations weakened the DNA–histone interactions in the region of the nucleosome dyad axis (region (+60)–(+80), Fig. 1) and substantially reduce the nucleosomal barrier at position (+45) of nucleosomal DNA [39]. The findings make it possible to assume that the barrier arises when the front Pol II edge, which moves approximately 10–20 bp ahead of the AC, occurs in nucleosomal DNA region (+60)–(+80) with strong DNA–histone interactions, while the Pol II AC is in the (+45) site.

Thus, the DNA–histone interactions in nucleosomal DNA region (+60)–(+80) determine, to a substantial extent, the strength of the nucleosomal barrier arising during transcription by Pol II. Similar results were obtained when DNA–histone interactions were studied by unzipping nucleosomal DNA with a laser "pincer" [40]. The experiments identified three regions of strong DNA–histone interactions: (+25)–(+35), (+60)–(+80), and (+115)–(+125). By analogy with the (+45) barrier, the nucleosomal barrier in the (+15) site is most likely caused to strong DNA–histone interactions in region (+25)–(+35) (Fig. 1).

As mentioned above, the strength of the (+15) and (+45) barriers only partly depends on the DNA sequence in regions (+25)–(+35) and (+60)–(+80), which are 10–20 bp upstream of the Pol II AC. At the same time, mutations of nucleosomal DNA region (+85)–(+95) substantially affect the strength of the (+45) nucleosomal barrier [41]. These experiments identified the additional nucleosomal DNA region (+85)–(+95) (hereafter referred to as a high affinity (HA) region) that determines the high (+45) nucleosomal barrier to transcription by Pol II. In contrast to

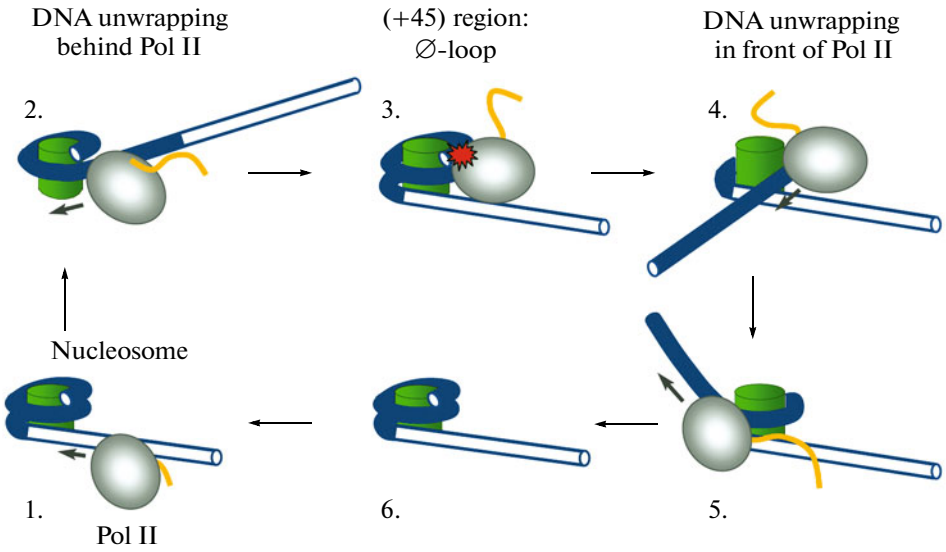

Fig. 2. Mechanism of Pol II transcription through the nucleosome. Complex 1: Pol II (gray oval) and the histone octamer (green cylinder) are shown; the transcription direction is indicated with small arrows. Complex 2: Pol II approaches the nucleosome, and DNA is partly unwrapped from the octamer behind the enzyme. Complex 3: Pol II encounters strong DNA–histone interactions during its further progress. An intranucleosomal ∅-loop forms in DNA to facilitate both histone preservation on the template and DNA unwrapping in front of the enzyme. Complex 4: DNA is further unwrapped, and transcription continues. Complex 5: Transcription through the nucleosome is complete, DNA–histone contacts re-form after the enzyme has moved further, and the nucleosome is restored in its initial position on DNA (complex 6). A temporal displacement of the H2A/H2B dimer is omitted.

the nucleosomal DNA regions mapped to positions (+25)–(+35) and (+60)–(+80), the HA region is more than 40 bp upstream of the Pol II AC, and the barrier strength depends on the DNA sequence to a great extent. The findings suggest an additional mechanism regulating transcription by Pol II; i.e., certain DNA sequences may further strengthen the DNA–histone interactions and thereby create an extremely high (and possibly regulated) barrier to the enzyme.

MOLECULAR MECHANISMS OF Pol II TRANSCRIPTION

How does Pol II overcome the nucleosomal barrier during transcription? A scheme that summarizes the results of long-term studies is shown in Fig. 2. As Pol II (complex 1) approaches and enters the nucleosome (complex 2), the DNA region behind the enzyme is partly unwrapped from the histone octamer [41]. After passing the (+45) site, Pol II encounters regions (+60)–(+80) and (+85)–(+95) with strong DNA–histone interactions (Fig. 1). These DNA regions are unwrapped and the barrier is overcome owing to the formation of an intranucleosomal DNA loop of an extremely small size at position (+49). The loop is known as the zero-size loop, or ∅-loop, in view of its size (Fig. 2, complex 3; the ∅-loop structure is shown in Fig. 3). The complex preserves the original pretranscriptional DNA–histone interactions in both the region upstream of the transcribing enzyme and the region that the enzyme has already passed.

Several features of the elongation complex (EC) with the ∅-loop were revealed in an analysis of its high-resolution model (Fig. 3) [41]. First, a major portion of the Pol II complex is exposed to the solution without steric clashes with the core histone molecules. Second, DNA is bent towards the octamer surface by 90° in the EC, and the bend facilitates the formation of the ∅-loop. Third, DNA–histone contacts formed behind the EC (in a DNA region of approximately 20 bp) stabilize the ∅-loop. Fourth, a displacement of ≥50 bp from the nucleosome end distal to the promoter reduces the DNA region interacting with histones in front of the nucleosome from approximately 100 to ≤50 bp. This should facilitate a further DNA unwrapping from the histone octamer surface in front of Pol II, as well as further transcription through the nucleosome. Finally, the HA sequence is within the displaced region (50 bp of nucleosomal DNA) and, as expected, is capable of preventing its displacement and blocking Pol II in the (+45) region.

Loop formation (a step limiting the nucleosome transcription rate) is slow and causes a characteristic pausing of Pol II at position (+45) [41]. It is important to note that the structure of the EC (+49) (i.e., the EC where the Pol II AS is at position (+49)) is incompatible with the presence of two turns in nucleosomal DNA, and one turn must be displaced. The formation of the ∅-loop results in a steric displacement and unwrapping of nucleosomal DNA in front of the enzyme, thus facilitating further transcription (Fig. 2, complex 4). Then, the initial DNA–histone interac-

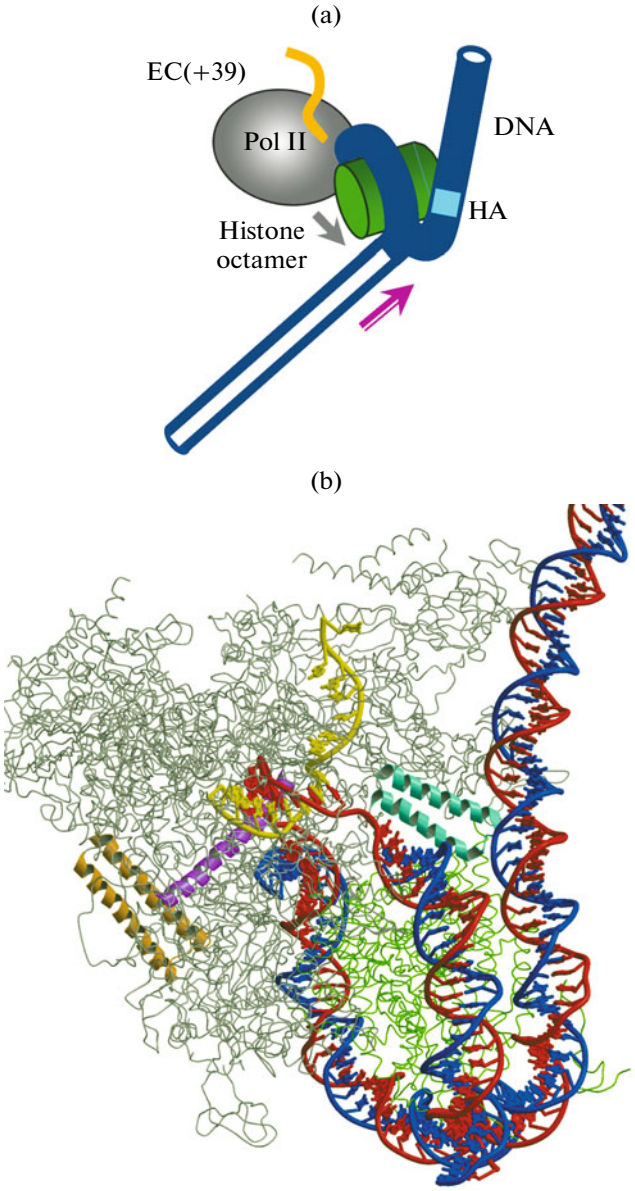

Fig. 3. Model of the intranucleosomal ∅-loop with Pol II. (a) EC(+39) forms after transcription of the first 39 bp of nucleosomal DNA. The DNA–histone contacts characteristic of the intact nucleosome (before transcription) form in front of an behind the transcribing enzyme to produce the ∅-loop. A 50-bp DNA region dissociates from the octamer surface. The nucleosome edge proximal to the promoter (pink arrow) and the transcription direction (gray arrow) are indicated. HA, a nucleosomal DNA region whose affinity for the histone octamer determines the strength of the (+45) nucleosomal barrier to a substantial extent. (b) Interposition of the nucleosome and yeast Pol II elongation complex structures (PDB IDs 1aoi and 1y1w, respectively) so that the Pol II AS is at position (+39). The template DNA, complementary DNA, and RNA strands are shown red, blue, and yellow, respectively.

tions form again (complex 5), and the structure of the nucleosome is restored after Pol II has passed it (complex 6).

The ∅-loop forms at least twice, in positions (+39) and (+49), during transcription through a nucleosome [39, 41]. The formation of small intranucleosomal, topologically closed DNA loops leads to an accumulation of positive supercoiling in front of Pol II as a result of Pol II rotation within a loop. This may provide a key mechanism that unwraps the histone octamer during transcription. A model of the EC (+49) was constructed using high-resolution structures (Fig. 3) and showed that the ∅-loop can only form at a certain rotational position of Pol II where a major part of the molecule is exposed to the solution, there is no steric clash with the core histones, and DNA is at 90° to the octamer. Moving by 1 bp, Pol II rotates around the DNA axis by ~36° and clashes against the histone octamer. The ∅-loop complex is stabilized by electrostatic Pol II–histone interactions, which arise in place of the DNA contacts with histones (approximately 20 bp) (Fig. 4). An analysis of the charge distribution on the octamer–Pol II interaction interface revealed a high negative charge on the Pol II surface in the immediate vicinity of a positively charged region on the surface of the histone octamer [41]. The region is within the core part of the clamp domain of the Pol II subunit RPB1, ensures the electrostatic interactions with the octamer [41], and temporally compensates for the disruption of the DNA–histone interactions in the nucleosome during transcription. The formation of the ∅-loop most likely helps to overcome the nucleosomal barrier during Pol II transcription [41].

Two models, for Pol II and Pol III, were advanced to explain the molecular mechanisms of transcript elongation in chromatin (table). The first mechanism is characterized by a high nucleosomal barrier to Pol II, eviction or exchange of histones H2A/H2B, and preservation of the nucleosome positions after transcription, as described above. The other mechanism is observed with Pol III and bacteriophage SP6 RNA polymerase and is characterized by a within-template transfer of the histone octamer, no eviction or loss of histones H2A/H2B, and a relatively weak nucleosomal barrier [42–45].

The intermediates that form during transcription through the nucleosome via the Pol III-type mechanism are well characterized structurally [42–44, 46, 47]. A comparison of the structures forming during chromatin transcription via the Pol II- and Pol III-type mechanisms is shown in Fig. 5. Similar structures form initially as Pol II or Pol III enters the nucleosome; in particular, DNA behind the elongation complex is separated from the surface of the histone octamer (Fig. 5; intermediates 2, 2'). DNA is partly detached from the octamer surface in front of the enzyme during Pol III transcription, but this most likely does not take place during Pol II transcription. Thus, great part of the octamer surface is exposed to the solution at early steps of Pol III transcription. This structural difference may explain why nucleosome translocation is more probable during Pol III tran-

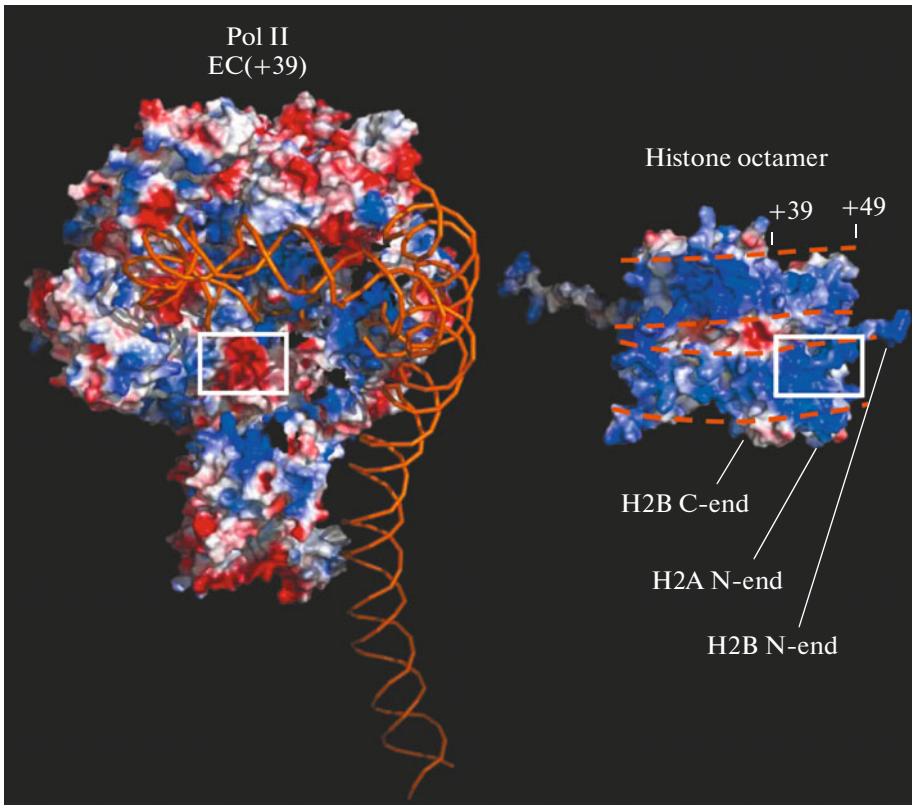

Fig. 4. Charge distribution on the contacting surfaces of Pol II and the histone octamer in the EC(+39) model. The contacting surfaces are shown for Pol II in EC(+39) (on the left, the histone octamer is omitted) and the histone octamer (on the right). The darkest blue and darkest red regions correspond to a potential of 82.3 and –82.3 kT/e, respectively. Regions that have opposite electrostatic charges and are in the immediate proximity in EC(+39) are framed. DNA (on the left) and its position in the intact nucleosome (on the right) are shown orange. Positions (+39) and (+49) of nucleosomal DNA and the positions of certain N-terminal histone tails are indicated. The nucleosome and Pol II structures (PDB IDs 1aoi and 1y1w, respectively) were used as a basis.

scription [42, 43, 45]. Accordingly, only 30% of all transcribed templates form complexes containing the ∅-loop [44]. In contrast, when DNA is not unwrapped from the surface of the histone octamer, the ∅-loop formation and the associated displacement of the promoter-distal end of nucleosomal DNA are more efficient. The ∅-loop allows DNA to preserve its original interactions with the octamer, and the nucleosomes consequently remain in their original positions on DNA after Pol II transcription. Thus, a more efficient formation of the intranucleosomal ∅-loop is characteristic of the Pol II-type mechanism and determines its other features.

ROLES OF THE H2A/H2B DIMERS DURING TRANSCRIPTION

One H2A/H2B dimer is lost as one Pol II complex passes through the nucleosome [48]. Accordingly, 50–95% of nucleosomes survive as hexasomes remaining in their original positions during Pol II transcription in vitro [48]. Histones H2A/H2B are evicted or displaced during the critical period of Pol II pausing at position (+45) [41]. As mentioned above, the period is associated with the formation of the ∅-loop, while DNA–histone interactions form behind the enzyme and DNA dissociates from the histone octamer in front of the enzyme, the two processes occurring in a coordinate manner. The nucleosomes are preserved in their initial positions owing to the formation of these temporal intranucleosomal loops [41].

Virtually identical as they are, the two H2A/H2B dimers play different roles in transcription through the nucleosome and differ in the consequences of their loss for transcription [41]. A removal of the distal (D) H2A/H2B dimer releases the promoter-distal end of nucleosomal DNA into the solution and facilitates the ∅-loop formation and transcription through the nucleosome [41]. An opposite effect is observed when the proximal (P) H2A/H2B dimer is removed; i.e., its displacement arrests Pol II in the nucleosome [41]. Thus, the P dimer is essential for nucleosome survival and efficient transcription. Dissociation of the P dimer most likely removes the DNA-binding site, which remains behind the EC in the (+45) position, thus destabilizing the ∅-loop structure. As noted

Characteristics of the Pol III- and Pol II-type transcription mechanisms

Activity	Enzyme	
	Pol III/SP6	Pol II/*E. coli*
Nucleosome relocation*	+	–
Displacement of H2A/H2B dimer	–	+
Strength of nucleosomal barrier	+	+++

* Nucleosomes are relocated in the direction opposite to that of the enzyme progress during transcription.

above, if the ∅-loop does not form, DNA is not unwrapped from the octamer before Pol II, while the unwrapping is necessary for the further progress of the enzyme.

ROLE OF THE FACT HISTONE CHAPERONE DURING TRANSCRIPTION

Highly purified experimental in vitro systems, which include only the Pol II EC, reproduce the important properties observed for transcribed chromatin in vivo and make it possible to study the mechanism of transcription through nucleosomes. However, the work of these systems is not optimal. Transcribing chromatin in vivo, the enzymatic complex progresses at a high rate and efficiently overcomes the nucleosomal barriers. It is clear that additional factors facilitate chromatin transcription in vivo and help the nucleosomes to preserve their positions during transcription. Transcribed genes are indeed associated in the cell with many factors, including ATP-dependent chromatin remodeling factors, transcription factors, histone chaperones, and histone-modifying enzymes ([49, 50], see below). Several complexes were found to facilitate chromatin transcription in vivo (TFIIS, FACT (Facilitates Chromatin Transcription), nucleolin, and multiple Pol II molecules). FACT is the most efficient in ensuring transcription of nucleosomal templates in a minimal in vitro system.

FACT is a heterodimeric protein complex that consists of two subunits (Spt16 and SSRP1) and acts as a transcription and replication factor and a chaperone for core histones [51–54]. FACT binds with the H2A/H2B dimer with higher affinity than with the H3/H4 tetramer [51, 55–57] and increases the chromatin transcription rate in vitro [37, 51, 53, 58]. In vivo, FACT colocalizes with Pol II and is similar to Pol II in the kinetic of binding to chromatin [59]. FACT is necessary for maintaining the chromatin structure during transcript elongation by Pol II [60, 61]. FACT facilitates gene transcription in regions with a highly ordered chromatin structure owing to its nucleosome-remodeling activity, which destabilizes the nucleosome structure and thereby promotes the progress of RNA polymerases [62]. Several hypotheses were advanced to describe the effect of FACT in chromatin. One hypothesis suggests that FACT promotes dissociation of one of the H2A/H2B histone dimers from the nucleosome to facilitate transcription [51]. According to another hypothesis, FACT improves the accessibility of nucleosomal DNA without displacing the H2A/H2B dimer [63, 64]. The hypotheses are

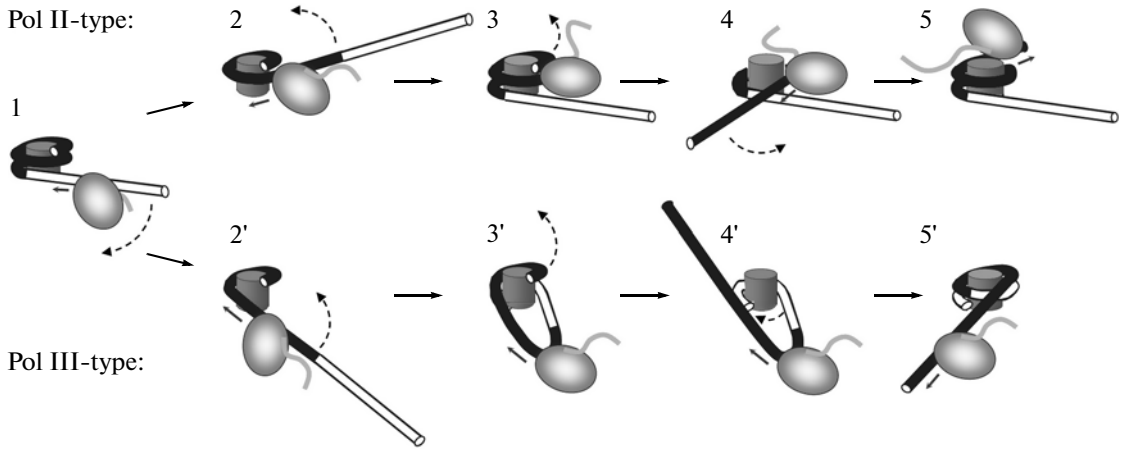

Fig. 5. Hypothetical mechanisms of Pol II- and Pol III-type transcription through chromatin. (1) Entering the nucleosome, (2, 2') the polymerases partly displace DNA from the histone octamer surface. However, a more extended DNA region downstream of the enzyme is displaced during transcription by the Pol III-type mechanism more efficiently than in the case of the Pol II-type mechanism, possibly because a higher rate is characteristic for Pol III-type transcription through nucleosomes). Thus, (3) the ∅-loop formation with Pol II is more efficient than with Pol II, and (3') larger intranucleosomal DNA loops tend to form in the latter case. (4, 4') The loop formation disrupts the DNA–histone interactions in front of the RNA polymerases, and then the nucleosome is restored (5) in the original position (in the case of Pol II) or (5') upstream of the original position (in the case of Pol III).

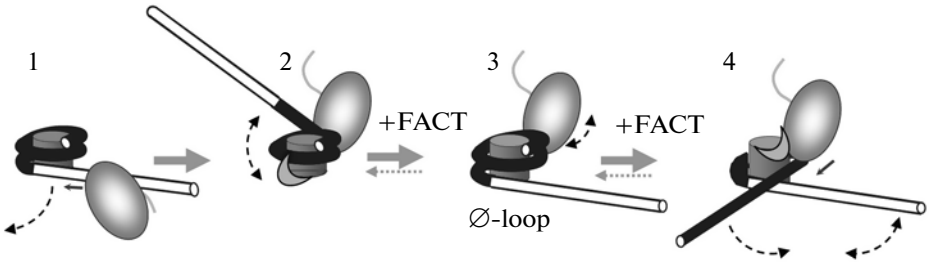

Fig. 6. Mechanism of FACT-dependent stimulation of transcription through the nucleosome. An individual FACT monomer is thought to interact with the DNA-binding surface of one H2A/H2B dimer after a partial unwrapping of nucleosomal DNA and to stimulate transcription by reducing the rate of subsequent dimer re-association with DNA. FACT initially interacts with the promoter-proximal dimer to facilitate transcription (complex 2). When position (+49) has been passed and nucleosomal DNA has partly dissociated from the histone octamer in front of Pol II, FACT interacts with the promoter-distal dimer to facilitate the formation of intermediate complex 4 and further transcription.

rather discrepant and still fail to explain the transcriptional effect of FACT in full. Our studies showed that FACT substantially reduces the nucleosomal barrier to Pol II transcription and that its effect fully depends on the presence of the C-terminal domain of the Spt16 subunit [51]. In addition, nucleosomes resulting from transcription in the presence of FACT lose one H2A/H2B dimer to become hexasomes [51], while new reaction products do not form.

Our experiments demonstrated additionally that stimulation of chromatin transcription by human FACT depends on the presence of the H2A/H2B dimers in the nucleosome [65]. As a kinetic analysis showed, FACT reduces the life of unproductive (arrested in the nucleosome) Pol II complexes and facilitates the formation of productive complexes, which contain DNA partly unwrapped from the octamer surface. In addition, the relative amount of free DNA resulting from transcription in the presence of FACT is far lower than in its absence [65]. FACT most likely interacts with the DNA-binding surface of the histone H2A/H2B dimers to facilitate partial spontaneous dissociation of nucleosomal DNA from the octamer.

The following model describes the FACT action during chromatin transcription by Pol II (Fig. 6) [65]. FACT presumably interacts with the DNA-binding surface of one of the two H2A/H2B dimers after partial dissociation of nucleosomal DNA from the octamer surface and facilitates transcription by reducing the rate of subsequent reassociation of the dimer with DNA (Fig. 6). The model suggests that hexasome survival during chromatin transcription is ensured by the efficient ∅-loop formation, which depends on the presence of the promoter-proximal H2A/H2B dimer, as described in detail above. FACT interacts with this dimer (Fig. 6, intermediate 2) to displace DNA. It is most likely that FACT additionally stabilizes the interactions of the H2A/H2B dimer with the other histones of the nucleosome and prevents the dimer from being displaced into the solution. After the ∅-loop has formed (Fig. 6, intermediate 3) and nucleosomal DNA has unwrapped from the surface of the promoter-distal H2A/H2B dimer, FACT interacts with the dimer and hinders DNA reassociation with the histone octamer (Fig. 6, intermediate 4). Thus, the interaction of FACT with the H2A/H2B dimers during transcription reduces the nucleosomal barrier and improves the efficiency of hexasome survival during transcription.

STUDIES OF TRANSCRIPTION BY MULTIPLE Pol II COMPLEXES

Nucleosomes survive when eukaryotic gene transcription by polymerase in vivo proceeds at a moderate rate, and only transcription-dependent exchange of histones H2A/H2B is observed in this case [17–19, 27, 28]. These parameters of transcribed chromatin are reproducible in the above in vitro systems, where single Pol II complexes transcribe nucleosomes and one transcription round occurs. At the same time, nucleosomes are partly removed and all of the core histones are exchanged when the same genes are transcribed in vivo at a higher rate [6, 17–26]. We assumed that the majority of the above parameters of transcribed chromatin are determined by a higher density of transcribing Pol II complexes on the gene. To check the assumption, an experimental system was developed to allow transcription by multiple complexes formed with Pol II or *Escherichia coli* RNA polymerase (RNAP), which is known to utilize the Pol II-type transcription mechanism [66]. The interaction of nucleosomes with tandem RNAP complexes allows the barrier to be overcome more efficiently and increases the yield of full-length transcripts. It should be noted that a strong nucleosomal barrier that Pol II fails to overcome even in the presence of FACT is almost completely abolished during transcription with two RNAP complexes [67]. The following RNAP complex considerably increases the transcription efficiency, which becomes comparable with that of histone-free DNA, and improves the transcription rate of the leading complex during its progress through the

nucleosomal barrier. M. Wang and colleagues [68] reported similar data on the effect of multiple complexes on the chromatin transcription efficiency.

At least two models can be advanced to explain the nucleosome displacement from highly active genes. One suggests a nucleosome collision with two tandem RNAP complexes. In this case, the following complex prevents the restoration of DNA–octamer contacts upstream of the leading complex and, consequently, the re-formation of the nucleosome in its initial position (the ∅-loop does not form). The other model postulates that the following Pol II complex displaces the hexasome that has formed behind the leading complex from DNA. The model does not require a simultaneous collision of the nucleosome with two Pol II complexes.

To check the former model, transcription was studied with one complex or a tandem of two complexes formed with *E. coli* RNAP or yeast Pol II [67]. A tandem EC, which consisted of two RNAP complexes progressing immediately one after the other, passed through nucleosomes more efficiently than single complexes. At the same time, transcription by the tandem EC did not increase the proportions of nucleosome-free DNA and hexasomes remaining on DNA after transcription.

According to the second model, several Pol II complexes encounter one nucleosome consecutively so that the following Pol II complex collides with the hexasome formed after transcription by the leading Pol II complex. An experimental verification of the model showed that all of the core histones are displaced from hexasomes in this case, including both hexasomes formed in the previous transcription round and those preexisting on the template. The key intermediate (especially the ∅-loop) of transcription through hexasomes are probably less stable than the intermediates of transcription through complete nucleosomes [67] (Fig. 7). Indeed, every histone H2A/H2B dimer stabilizes approximately a 35-bp nucleosomal DNA region. Entering the nucleosome, Pol II disrupts the DNA–histone interactions behind the transcription complex before reaching position (+49). Accordingly, fewer DNA–histone interactions stabilize the Pol II • EC(+45) complex with the nucleosome that lacks the promoter-distal H2A/H2B dimer, which has been displaced by the previous transcribing Pol II complex (Fig. 7, complex 2'), and the histone hexamer can spontaneously dissociate into the solution from this complex. The data agree with the histone exchange or eviction observed during intense transcription in vivo [21–26, 69, 70].

The results obtained in vitro with multiple Pol II complexes make it possible to assume that the following complex encounters the hexasome that has formed as a result of the previous transcription round before a reassociation of the H2A/H2B dimer. A hexasome is an unstable intermediate form of the nucleosome with fewer DNA–histone contacts. When a hexasome collides with a next RNAP, all of the core histones are removed from DNA in vitro. The model explains the fact that histones H3/H4 remain on DNA at a moderate transcription rate and are displaced from actively transcribed genes. Moreover, the model explains why the histone displacement efficiency depends on the transcription efficiency in vivo [17–19, 24], suggesting that the histone displacement efficiency is inversely proportional to the mean distance between transcribing complexes.

MECHANISMS OF Pol II TRANSCRIPTION THROUGH CHROMATIN in vivo

In vivo, Pol II transcription stops soon after its initiation on the promoter with thousands of genes of higher organisms from *Drosophila* to human [9–11] (Fig. 8, (1)). The nascent RNA associated with the enzyme is no more than 100 nt in this case [71–75]. The first nucleosome located downstream of the transcription start ((+1) nucleosome) provides a strong barrier for Pol II and is potentially involved in regulating transcription in eukaryotes. When protein factors activate transcription on the promoter, the barrier is overcome, and Pol II continues synthesizing the RNA strand. The barrier can be overcome with the help of the elongation factor TFIIS, FACT, chromatin-remodeling complexes; a removal of the DSIF and NELF repressors; or acetylation of the N-terminal histone tails. In addition, two tandem Pol II molecules successfully overcome the (+1) nucleosomal barrier in vitro, and the transcription-stimulating effect of the tandem is greater than that of any of the factors (see above). Because the site of the polymerase arrest is quite typical (the AC of the enzyme is 30–50 bp downstream of the transcription start [76]), it is possible to assume that two tandem Pol II ECs come close together on such genes to overcome the (+1) nucleosomal barriers and other hindrances (e.g., DNA-binding proteins) to Pol II progress [67, 68, 77, 78]. A Pol II tandem may additionally act as a pioneering enzyme that modifies the chromatin structure of the transcribed gene to improve its accessibility for further transcription.

Once the initial nucleosomal barrier is overcome (possibly, with the help of a second Pol II complex and the above factors), Pol II can continue transcription at a rate of approximately 3–4 kb/min [11, 12], which also requires several factors, such as ATP-dependent chromatin-remodeling factors, transcription factors, histone chaperones, and histone-modifying enzymes [49, 50]. The FACT and Asf1 histone chaperones are associated with transcribed genes and facilitate nucleosome assembly–disassembly during elongation [59, 80]. Histone acetylation promotes transcription through chromatin and transcription-dependent histone exchange, whereas methylation leads to a binding of histone deacetylases and a restoration of the inactive chromatin state after transcription [49, 81, 82].

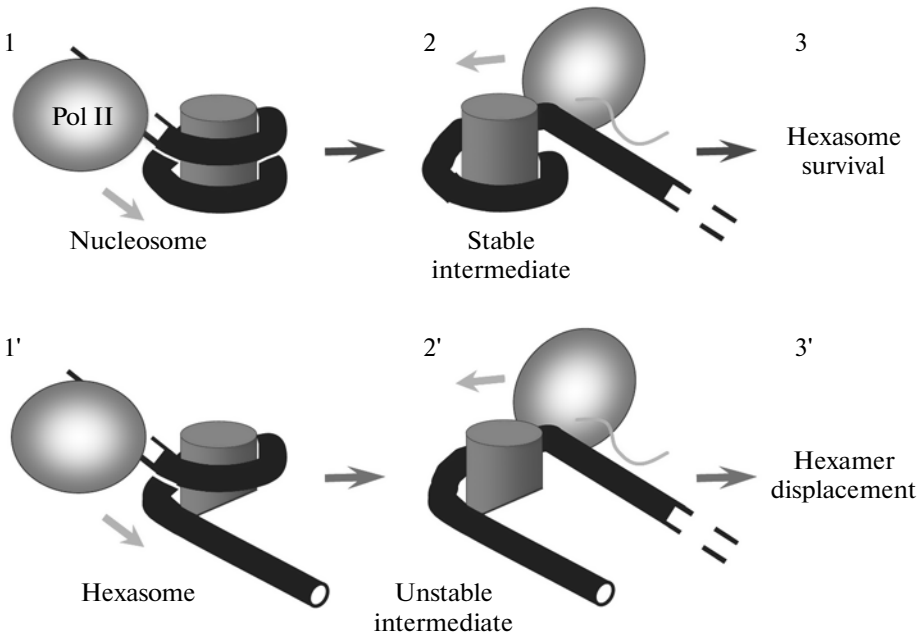

Fig. 7. Nucleosome survival during transcription depends on the distance between Pol II complexes. Intermediates 1, 2, and 3: mechanism of nucleosome survival during transcription. As Pol II (1) approaches and (2) enters the nucleosome, it partly displaces DNA from the histone octamer surface. (3) However, the DNA region in front of Pol II remains bound to the octamer, ensuring the formation of a stable intermediate complex and the preservation of the hexasome in its original position. In contrast, (1') transcription through the hexasome resulting from loss of one H2A/H2B dimer proceeds via (2') an unstable intermediate complex with fewer DNA–histone contacts, (3') leading to a displacement of the histone hexamer from DNA.

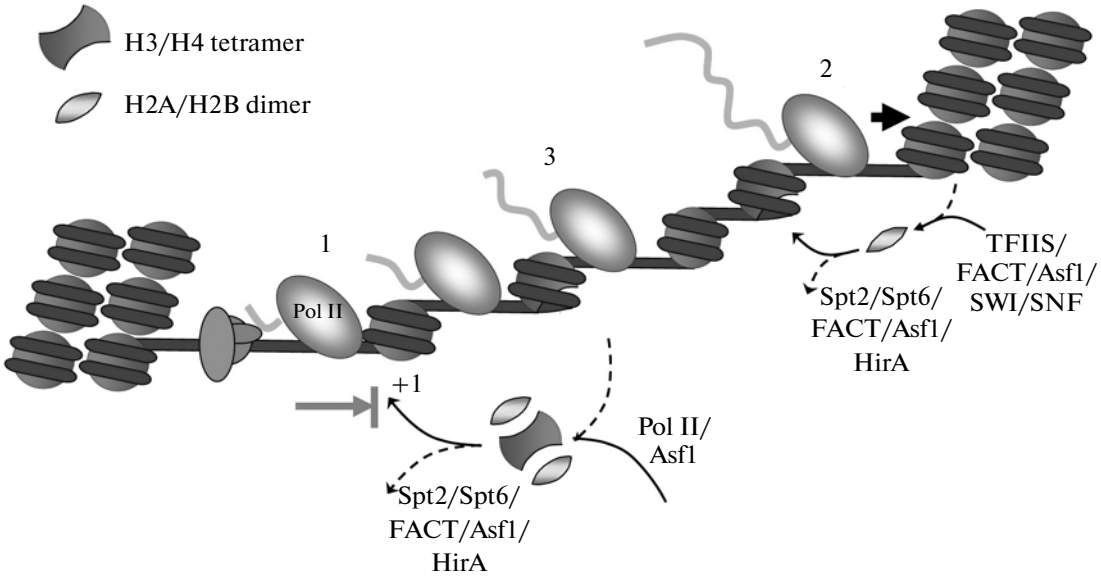

Fig. 8. Mechansim of Pol II transcription through chromatin in vivo. (1) After transcription has been initiated on the promoter, Pol II pauses once entering the (+1) nucleosome in many human and *Drosophila* genes. When the (+1) nucleosomal barrier is overcome, further transcription by (2) single or (3) multiple tandem Pol II complexes is accompanied by eviction and exchange of histones H2A/H2B or all of the core histones, respectively. At a low Pol II density, only the H2A/H2B dimer is temporally evicted and exchanged, and the nucleosome structure is restored before the next Pol II complex arrives. At a high Pol II density, Pol II complexes encounter the hexasomes lacking the H2A/H2B dimers, and all of the core histones are displaced and exchanged. Several factors that interact with chromatin and are associated with transcribed genes are indicated.

Acetyltransferases PCAF and Elp3, which modify the histone tails, specifically interacts with phosphorylated elongating Pol II [50, 83], and NuA3 interacts with the FACT elongation factor both in vitro and in vivo [84]. Ubiquitination of histone H2B and the CHD1 chromatin-remodeling enzymes are necessary for the recruitment of the Spt16 protein factor to transcribed genes, efficient Pol II transcription, and nucleosome assembly [85–87]. In addition, acetylated H3K56 is associated with transcribing Pol II [88].

The above factors, along with Pol II, are involved in several probable scenarios of gene transcription in vivo (Fig. 8). At a low to moderate rate of transcription by single enzyme molecules, which are spatially distant, the process is accompanied by a temporal eviction or exchange of the H2A/H2B dimer(s) [24, 25], and the nucleosomes survive in the form of hexasomes as a result of the formation of small intranucleosomal DNA loops (Fig. 8, (2)). The H2A/H2B dimer then binds to the hexasome, and the initial nucleosome structure is restored before the chromatin region is transcribed with the next Pol II complex. Histones H3/H4 are known to bear the majority of posttranslational modifications, including certain epigenetic marks. Therefore, the Pol II-type transcription mechanism ensures the preservation of the original histones H3/H4 and their covalent modifications during transcription.

At a high transcription rate, the distance between transcribing Pol II complexes is shorter, and the complexes encounter the hexasomes lacking the H2A/H2B dimer (Fig. 8, (3)). An unstable intermediate with fewer DNA–histone contacts forms in this case, leading to eviction from DNA and exchange of all of the histones [21–26, 69, 70].

CONCLUSIONS

The mechanism of Pol II transcription through chromatin is conserved among various species from yeast to human and consists in a strongly regulated alternation of temporal disruption and restoration of DNA–histone and histone–histone interactions. Defects in these processes and irreversible loss of nucleosomes impair the cell viability and are often associated with pathology [33–36]. Hence, understanding the detailed mechanism of transcription through chromatin is not only of interest as a subject of basic research, but it is of applied significance for designing new drugs, which may find application in therapy of oncology and age-related diseases.

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REFERENCES

1. Thuriaux P., Sentenac A. 1992. In: *Molecular and Cellular Biology of the Yeast Saccharomyces: Gene Expression*. Jones E.W., Pringle J.R., Broach J.R. Eds., Cold Spring Harbor, NY: Cold Spring Harbor Laboratory Press, vol. 2, pp. 1–8.
2. Gnatt A.L., Cramer P., Fu J., Bushnell D.A., Kornberg R.D. 2001. Structural basis of transcription: An RNA polymerase II elongation complex at 3.3 Å resolution. *Science*. **292**, 1876–882.
3. Boeger H., Griesenbeck J., Kornberg R.D. 2008. Nucleosome retention and the stochastic nature of promoter chromatin remodeling for transcription. *Cell*. **133**, 716–726.
4. Kim H.D., O'Shea E.K. 2008. A quantitative model of transcription factor activated gene expression. *Nature Struct. Mol. Biol*. **15**, 1192–1198.
5. Lam F.H., Steger D.J., O'Shea E.K 2008. Chromatin decouples promoter threshold from dynamic range. *Nature*. **453**, 246–250.
6. Petesch S.J., Lis J.T. 2008. Rapid, transcription-independent loss of nucleosomes over a large chromatin domain at Hsp70 loci. *Cell*. **134**, 74–84.
7. Whitehouse I., Rando O.J., Delrow J., Tsukiyama T. 2007. Chromatin remodelling at promoters suppresses antisense transcription. *Nature*. **450**, 1031–1035.
8. Zhao X., Pendergrast P.S., Hernandez N. 2001. A positioned nucleosome on the human U6 promoter allows recruitment of SNAPc by the Oct1 POU domain. *Mol. Cell*. **7**, 539–549.
9. Guenther M.G., Levine S.S., Boyer L.A., Jaenisch R., Young R.A. 2007. A chromatin landmark and transcription initiation at most promoters in human cells. *Cell*. **130**, 77–88.
10. Zeitlinger J., Stark A., Kellis M., Hong J.W., Nechaev S., Adelman K., Levine M., Young R.A. 2007. RNA polymerase stalling at developmental control genes in the *Drosophila melanogaster* embryo. *Nature Genet*. **39**, 1512–1516.
11. Mavrich T.N., Jiang C., Ioshikhes I.P., et al. 2008. Nucleosome organization in the *Drosophila* genome. *Nature*. **453**, 358–362.
12. Churchman L.S., Weissman J.S. 2011. Nascent transcript sequencing visualizes transcription at nucleotide resolution. *Nature*. **469**, 368–373.
13. Gilchrist D.A., Dos Santos G., Fargo D.C., Xie B., Gao Y., Li L., Adelman K. 2010. Pausing of RNA polymerase II disrupts DNA-specified nucleosome organization to enable precise gene regulation. *Cell*. **143**, 540–551.
14. Singh J., Padgett R.A. 2009. Rates of in situ transcription and splicing in large human genes. *Nature Struct. Mol. Biol*. **16**, 1128–1133.
15. Cheng B., Price D.H. 2007. Properties of RNA polymerase II elongation complexes before and after the P-TEFb-mediated transition into productive elongation. *J. Biol. Chem*. **282**, 21901–12912.

16. Izban M.G., Luse D.S. 1992. Factor-stimulated RNA polymerase II transcribes at physiological elongation rates on naked DNA but very poorly on chromatin templates. *J. Biol. Chem*. **267**, 13647–13655.
17. Kristjuhan A., Svejstrup J.Q. 2004. Evidence for distinct mechanisms facilitating transcript elongation through chromatin in vivo. *EMBO J*. **23**, 4243–4252.
18. Lee C.K., Shibata Y., Rao B., Strahl B.D., Lieb J.D. 2004. Evidence for nucleosome depletion at active regulatory regions genome-wide. *Nature Genet*. **36**, 900–905.
19. Schwabish M.A., Struhl K. 2004. Evidence for eviction and rapid deposition of histones upon transcriptional elongation by RNA polymerase II. *Mol. Cell. Biol*. **24**, 10111–10117.
20. Zhao J., Herrera-Diaz J., Gross D.S. 2005. Domain-wide displacement of histones by activated heat shock factor occurs independently of Swi/Snf and is not correlated with RNA polymerase II density. *Mol. Cell. Biol*. **25**, 8985–8999.
21. Wirbelauer C., Bell O., Schubeler D. 2005. Variant histone H3.3 is deposited at sites of nucleosomal displacement throughout transcribed genes while active histone modifications show a promoter-proximal bias. *Genes Dev*. **19**, 1761–1766.
22. Schwartz B.E., Ahmad K. 2005. Transcriptional activation triggers deposition and removal of the histone variant H3.3. *Genes Dev*. **19**, 804–814.
23. Thiriet C., Hayes J.J. 2005. Replication-independent core histone dynamics at transcriptionally active loci in vivo. *Genes Dev*. **19**, 677–582.
24. Dion M.F., Kaplan T., Kim M., Buratowski S., Friedman N., Rando O.J. 2007. Dynamics of replication-independent histone turnover in budding yeast. *Science*. **315**, 1405–1408.
25. Rufiange A., Jacques P.E., Bhat W., Robert F., Nourani A. 2007. Genome-wide replication-independent histone H3 exchange occurs predominantly at promoters and implicates H3 K56 acetylation and Asf1. *Mol. Cell*. **27**, 393–405.
26. Jamai A., Imoberdorf R.M., Strubin M. 2007. Continuous histone H2B and transcription-dependent histone H3 exchange in yeast cells outside of replication. *Mol. Cell*. **25**, 345–355.
27. Nacheva G.A., Guschin D.Y., Preobrazhenskaya O.V., Karpov V.L., Ebralidse K.K., Mirzabekov A.D. 1989. Change in the pattern of histone binding to DNA upon transcriptional activation. *Cell*. **58**, 27–36.
28. Jiang C., Pugh B.F. 2009. Nucleosome positioning and gene regulation: Advances through genomics. *Nature Rev. Genet*. **10**, 161–172.
29. Chen T.A., Allfrey V.G. 1987. Rapid and reversible changes in nucleosome structure accompany the activation, repression, and superinduction of murine fibroblast protooncogenes *c-fos* and *c-myc*. *Proc. Natl. Acad. Sci. U. S. A*. **84**, 5252–5256.
30. Chen T.A., Smith M.M., Le S.Y., Sternglanz R., Allfrey V.G. 1991. Nucleosome fractionation by mercury affinity chromatography. Contrasting distribution of transcriptionally active DNA sequences and acetylated histones in nucleosome fractions of wild-type yeast cells and cells expressing a histone H3 gene altered to encode a cysteine 110 residue. *J. Biol. Chem*. **266**, 6489–6498.
31. Prior C.P., Cantor C.R., Johnson E.M., Littau V.C., Allfrey V.G. 1983. Reversible changes in nucleosome structure and histone H3 accessibility in transcriptionally active and inactive states of rDNA chromatin. *Cell*. **34**, 1033–1042.
32. Walia H., Chen H.Y., Sun J.M., Holth L.T., Davie J.R. 1998. Histone acetylation is required to maintain the unfolded nucleosome structure associated with transcribing DNA. *J. Biol. Chem*. **273**, 14516–14522.
33. Martens J.A., Wu P.Y., Winston F. 2005. Regulation of an intergenic transcript controls adjacent gene transcription in *Saccharomyces cerevisiae*. *Genes Dev*. **19**, 2695–2704.
34. Feser J., D.T., Das C., Carson J.J., Kieft J., Harkness T., Tyler J.K. 2010. Elevated histone expression promotes life span extension. *Mol. Cell*. **39**, 724–735.
35. Ljungman M., Hanawalt P.C. 1992. Efficient protection against oxidative DNA damage in chromatin. *Mol. Carcinog*. **5**, 264–269.
36. Enright H.U., Miller W.J., Hebbel R.P. 1992. Nucleosomal histone protein protects DNA from iron-mediated damage. *Nucleic Acids Res*. **20**, 3341–3346.
37. Bondarenko V.A., Steele L.M., Ujvari A., Gaykalova D.A., Kulaeva O.I., Polikanov Y.S., Luse D.S., Studitsky V.M. 2006. Nucleosomes can form a polar barrier to transcript elongation by RNA polymerase II. *Mol. Cell*. **24**, 469–479.
38. Muthurajan U.M., Bao Y., Forsberg L.J., Edayathumangalam R.S., Dyer P.N., White C.L., Luger K. 2004. Crystal structures of histone Sin mutant nucleosomes reveal altered protein–DNA interactions. *EMBO J*. **23**, 260–271.
39. Hsieh F.K., Fisher M., Ujvari A., Studitsky V.M., Luse D.S. 2010. Histone Sin mutations promote nucleosome traversal and histone displacement by RNA polymerase II. *EMBO Rep*. **11**, 705–710.
40. Hall M.A., Shundrovsky A., Bai L., Fulbright R.M., Lis J.T., Wang M.D. 2009. High-resolution dynamic mapping of histone–DNA interactions in a nucleosome. *Nature Struct. Mol. Biol*. **16**, 124–129.
41. Kulaeva O.I., Gaykalova D.A., Pestov N.A., Golovastov V.V., Vassylyev D.G., Artsimovitch I., Studitsky V.M. 2009. Mechanism of chromatin remodeling and recovery during passage of RNA polymerase II. *Nature Struct. Mol. Biol*. **16**, 1272–1278.
42. Studitsky V.M., Clark D. J., Felsenfeld G. 1995. Overcoming a nucleosomal barrier to transcription. *Cell*. **83**, 19–27.
43. Studitsky V.M., Clark D.J., Felsenfeld G. 1994. A histone octamer can step around a transcribing polymerase without leaving the template. *Cell*. **76**, 371–382.
44. Bednar J., Studitsky V.M., Grigoryev S.A., Felsenfeld G., Woodcock C.L. 1999. The nature of the nucleosomal barrier to transcription: Direct observation of paused intermediates by electron cryomicroscopy. *Mol. Cell*. **4**, 377–386.
45. Studitsky V.M., Kassavetis G.A., Geiduschek E.P., Felsenfeld G. 1997. Mechanism of transcription through the nucleosome by eukaryotic RNA polymerase. *Science*. **278**, 1960–1963.

46. Studitsky V.M., Orlovsky I.V., Chertkov O.V., Efimova N.S., Loginova M.A., Kulaeva O.I. 2012. Molecular mechanisms of transcription through chromatin by RNA polymerase III: Part 1, *Vestn. Mosk. Gos. Univ. Ser. 16: Biol.* **3**, 6–11

47. Studitsky V.M., Orlovsky I.V., Chertkov O.V., Efimova N.S., Loginova M.A., Kulaeva O.I. 2012. Molecular mechanisms of transcription through chromatin by RNA polymerase III: Part 2, *Vestn. Mosk. Gos. Univ. Ser. 16: Biol.* **4**, 10–16.

48. Kireeva M.L., Walter W., Tchernajenko V., Bondarenko V., Kashlev M., Studitsky V.M. 2002. Nucleosome remodeling induced by RNA polymerase II: Loss of the H2A/H2B dimer during transcription. *Mol. Cell.* **9**, 541–552.

49. Selth L.A., Sigurdsson S., Svejstrup J.Q. 2010. Transcript elongation by RNA polymerase II. *Annu. Rev. Biochem.* **79**, 271–293.

50. Wittschieben B.O., Otero G., de Bizemont T., Fellows J., Erdjument-Bromage H., Ohba R., Li Y., Allis C.D., Tempst P., Svejstrup J.Q. 1999. A novel histone acetyltransferase is an integral subunit of elongating RNA polymerase II holoenzyme. *Mol. Cell.* **4**, 123–128.

51. Belotserkovskaya R., Oh S., Bondarenko V.A., Orphanides G., Studitsky V.M., Reinberg D. 2003. FACT facilitates transcription-dependent nucleosome alteration. *Science.* **301**, 1090–1093.

52. Wittmeyer J., Formosa T. 1997. The *Saccharomyces cerevisiae* DNA polymerase alpha catalytic subunit interacts with Cdc68/Spt16 and with Pob3, a protein similar to an HMG1-like protein. *Mol. Cell Biol.* **17**, 4178–4190.

53. Orphanides G., LeRoy G., Chang C.H., Luse D.S., Reinberg D. 1998. FACT, a factor that facilitates transcript elongation through nucleosomes. *Cell.* **92**, 105–116.

54. Orphanides G., Wu W.H., Lane W.S., Hampsey M., Reinberg D. 1999. The chromatin-specific transcription elongation factor FACT comprises human SPT16 and SSRP1 proteins. *Nature.* **400**, 284–288.

55. Stuwe T., Hothorn M., Lejeune E., Rybin V., Bortfeld M., Scheffzek K., Ladurner A G. 2008. The FACT Spt16 "peptidase" domain is a histone H3-H4 binding module. *Proc. Natl. Acad. Sci. U. S. A.* **105**, 8884–8889.

56. Winkler D.D., Luger K. 2011. The histone chaperone FACT: Structural insights and mechanisms for nucleosome reorganization. *J. Biol. Chem.* **286**, 18369–18374.

57. Winkler D.D., Muthurajan U.M., Hieb A.R., Luger K. 2011. Histone chaperone FACT coordinates nucleosome interaction through multiple synergistic binding events. *J. Biol. Chem.* **286**, 41883–41892.

58. Ujvari A., Hsieh F.K., Luse S.W., Studitsky V.M., Luse D.S. 2008. Histone N-terminal tails interfere with nucleosome traversal by RNA polymerase II. *J. Biol. Chem.* **283**, 32236–32243.

59. Saunders A., Werner J., Andrulis E.D., Nakayama T., Hirose S., Reinberg D., Lis J.T. 2003. Tracking FACT and the RNA polymerase II elongation complex through chromatin in vivo. *Science.* **301**, 1094–1096.

60. Mason P.B., Struhl K. 2003. The FACT complex travels with elongating RNA polymerase II and is important for the fidelity of transcriptional initiation in vivo. *Mol. Cell Biol.* **23**, 8325–8333.

61. Cheung V., Chua G., Batada N.N., Landry C.R., Michnick S.W., Hughes T.R., Winston F. 2008. Chromatin- and transcription-related factors repress transcription from within coding regions throughout the *Saccharomyces cerevisiae* genome. *PLoS Biol.* **6**, e277.

62. Reinberg D.S.R. 2006. de FACTo nucleosome dynamics. *J. Biol. Chem.* **281**, 23297–23301.

63. McCullough L., Rawlins R., Olsen A., Xin H., Stillman D.J., Formosa T. 2011. Insight into the mechanism of nucleosome reorganization from histone mutants that suppress defects in the FACT histone chaperone. *Genetics.* **188**, 835–846.

64. Xin H., Takahata S., Blanksma M., McCullough L., Stillman D.J., Formosa T. 2009. yFACT induces global accessibility of nucleosomal DNA without H2A-H2B displacement. *Mol. Cell.* **35**, 365–376.

65. Hsieh F.K., Kulaeva O.I., Patel S.S., Dyer P.N., Luger K., Reinberg D., Studitsky V.M. 2013. Mechanism of FACT action during Pol II transcription through chromatin. *Proc. Natl. Acad. Sci. U. S. A.* **110**, 7654–7659.

66. Walter W., Kireeva M.L., Studitsky V.M., Kashlev M. 2003. Bacterial polymerase and yeast polymerase II use similar mechanisms for transcription through nucleosomes. *J. Biol. Chem.* **278**, 36148–36156.

67. Kulaeva O.I., Hsieh F.K., Studitsky V.M. 2010. RNA polymerase complexes cooperate to relieve the nucleosomal barrier and evict histones. *Proc. Natl. Acad. Sci. U. S. A.* **107**, 11325–11330.

68. Jin J., Bai L., Johnson D.S., Fulbright R.M., Kireeva M.L., Kashlev M., Wang M.D. 2010. Synergistic action of RNA polymerases in overcoming the nucleosomal barrier. *Nature Struct. Mol. Biol.* **17**, 745–752.

69. Deal R.B., Henikoff J.G., Henikoff S. 2010. Genome-wide kinetics of nucleosome turnover determined by metabolic labeling of histones. *Science.* **328**, 1161–1164.

70. Katan-Khaykovich Y., Struhl K. 2011. Splitting of H3-H4 tetramers at transcriptionally active genes undergoing dynamic histone exchange. *Proc. Natl. Acad. Sci. U. S. A.* **108**, 1296–1301.

71. Core L.J., Lis J.T. 2008. Transcription regulation through promoter-proximal pausing of RNA polymerase II. *Science.* **319**, 1791–1792.

72. Price D.H. 2008. Poised polymerases: On your mark... Get set... Go! *Mol. Cell.* **30**, 7-10.

73. Nechaev S., Adelman K. 2008. Promoter-proximal Pol II: When stalling speeds things up. *Cell Cycle.* **7**, 1539–1544.

74. Gilmour D.S. 2009. Promoter proximal pausing on genes in metazoans. *Chromosoma.* **118**, 1–10.

75. Adelman, K., Lis J.T. 2012. Promoter-proximal pausing of RNA polymerase II: Emerging roles in metazoans. *Nature Rev. Genet.* **13**, 720–731.

76. Muse G.W., Gilchrist D.A., Nechaev S., Shah R., Parker J.S., Grissom S.F., Zeitlinger J., Adelman K. 2007. RNA polymerase is poised for activation across the genome. *Nature Genet.* **39**, 1507–1511.

77. Epshtein V., Nudler E. 2003. Cooperation between RNA polymerase molecules in transcription elongation. *Science*. **300**, 801–805.

78. Epshtein V., Toulme F., Rahmouni A.R., Borukhov S., Nudler E. 2003. Transcription through the roadblocks: The role of RNA polymerase cooperation. *EMBO J*. **22**, 4719–4727.

79. Gaykalova D.A., Vivekananthan N., Bondarenko V.A., Bartholomew B., Clark D.J., Studitsky V.M. 2010. A polar barrier to transcription is circumvented by remodeller-induced nucleosome translocation. *Nucleic Acids Res*. **39**, 3520–3528.

80. Schwabish M.A., Struhl K. 2006. Asf1 mediates histone eviction and deposition during elongation by RNA polymerase II. *Mol. Cell*. **22**, 415–422.

81. Carrozza M.J., Li B., Florens L., Suganuma T., Swanson S.K., Lee K.K., Shia W.J., Anderson S., Yates J., Washburn M.P., Workman J.L. 2005. Histone H3 methylation by Set2 directs deacetylation of coding regions by Rpd3S to suppress spurious intragenic transcription. *Cell*. **123**, 581–592.

82. Workman J.L. 2006. Nucleosome displacement in transcription. *Genes Dev*. **20**, 2009–2017.

83. Cho H., Orphanides G., Sun X., Yang X.J., Ogryzko V., Lees E., Nakatani Y., Reinberg D. 1998. A human RNA polymerase II complex containing factors that modify chromatin structure. *Mol. Cell. Biol*. **18**, 5355–5363.

84. John S., Howe L., Tafrov S.T., Grant P.A., Sternglanz R., Workman J.L. 2000. The something about silencing protein, Sas3, is the catalytic subunit of NuA3, a yTAF(II)30-containing HAT complex that interacts with the Spt16 subunit of the yeast CP (Cdc68/Pob3)-FACT complex. *Genes Dev*. **14**, 1196–11208.

85. Fleming A.B., Kao C.F., Hillyer C., Pikaart M., Osley M.A. 2008. H2B ubiquitylation plays a role in nucleosome dynamics during transcription elongation. *Mol. Cell*. **31**, 57–66.

86. Pavri R., Zhu B., Li G., Trojer P., Mandal S., Shilatifard A., Reinberg D. 2006. Histone H2B monoubiquitination functions cooperatively with FACT to regulate elongation by RNA polymerase II. *Cell*. **125**, 703–717.

87. Lee J.S., Garrett A.S., Yen K., Takahashi Y.H., Hu D., Jackson J., Seidel C., Pugh B.F., Shilatifard A. 2012. Codependency of H2B monoubiquitination and nucleosome reassembly on Chd1. *Genes Dev*. **26**, 914–919.

88. Schneider J., Bajwa P., Johnson F.C., Bhaumik S.R., Shilatifard A. 2006. Rtt109 is required for proper H3K56 acetylation: A chromatin mark associated with the elongating RNA polymerase II. *J. Biol. Chem*. **281**, 37270–37274.

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