# RNA polymerase complexes cooperate to relieve the nucleosomal barrier and evict histones

**Olga I. Kulaeva[1], Fu-Kai Hsieh[1], and Vasily M. Studitsky[2]**

Department of Pharmacology, University of Medicine and Dentistry of New Jersey–Robert Wood Johnson Medical School, Piscataway, NJ 08854

Edited by Gary Felsenfeld, National Institutes of Health, Bethesda, MD, and approved May 17, 2010 (received for review February 5, 2010)

**Maintenance of the chromatin states and histone modification patterns during transcription is essential for proper gene regulation and cell survival. Histone octamer survives moderate transcription, but is evicted during intense transcription in vivo by RNA polymerase II (Pol II). Previously we have shown that nucleosomes can survive transcription by single Pol II complexes in vitro. To study the mechanism of histone displacement from DNA, the encounter between multiple complexes of RNA polymerase and a nucleosome was analyzed in vitro. Multiple transcribing Pol II complexes can efficiently overcome the high nucleosomal barrier and displace the entire histone octamer, matching the observations in vivo. DNA-bound histone hexamer left behind the first complex of transcribing enzyme is evicted by the next Pol II complex. Thus transcription by single Pol II complexes allows survival of the original H3/H4 histones, while multiple, closely spaced complexes of transcribing Pol II can induce displacement of all core histones along the gene.**

elongation | rate | cooperation | subnucleosome | eviction

Efficient maintenance of nucleosomal organization during passage of RNA polymerase II (Pol II) is accompanied by recovery of nucleosomal structure—a process that is essential for proper gene regulation and cell survival (1, 2). The nucleosome recovery occurs through two different mechanisms (see refs. 3 and 4 for review). Only H2A/H2B, but not H3/H4, histones are exchanged on moderately transcribed genes (5–10). In contrast, during intense transcription, all core histones are evicted from DNA (11–13) and exchanged (5–10). These studies suggest that during Pol II transcription in vivo histones H2A/H2B are constitutively displaced/exchanged, whereas histones H3/H4 are displaced/exchanged only during intense transcription (3, 4).

Nucleosome recovery after Pol II transcription has been recapitulated in vitro using a single-round transcription assay. During nucleosome traversal by Pol II in vitro, a single H2A/H2B dimer is released (14–16). The displacement of H2A/H2B histones in vitro matches the apparent effect of Pol II passage in vivo during moderate transcription (7, 17). The subnucleosome (hexasome, DNA-bound histone hexamer) survives single-round transcription by Pol II in vitro (14) through transient formation of a small intranucleosomal DNA loop containing transcribing enzyme (18). The mechanism of histone eviction/exchange during intense transcription by multiple, closely spaced Pol II complexes remains unknown.

We have proposed a possible mechanism that could explain the observed displacement/exchange of H3/H4 histones during intense transcription by Pol II in vivo (4). It has been suggested that the presence of multiple Pol II complexes accumulated on a gene at high density during intense transcription induces displacement (eviction) of the entire histone octamer from DNA (Fig. 1*A*). In this work the key predictions of this model have been experimentally evaluated in vitro through analysis of the encounter between a nucleosome and a single or multiple complexes of transcribing RNAP. The presence of the second RNAP complex closely behind the first complex results in a strong increase in the efficiency of transcription through a nucleosomal barrier. Moreover, while the first complex of RNA polymerase displaces only one H2A/H2B dimer from DNA, the second complex can evict the entire histone hexamer remaining on the template. These data match the observations in vivo and suggest that the key

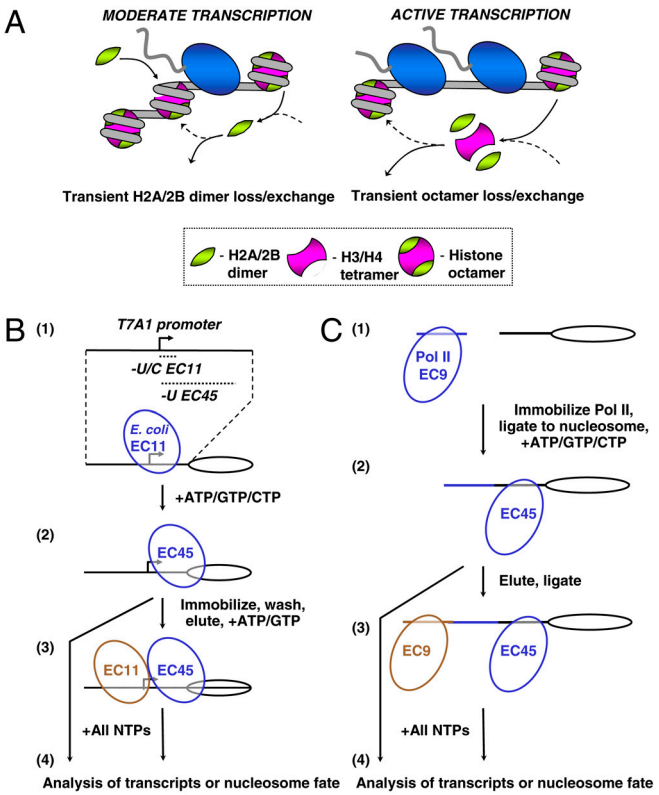


**Fig. 1.** Experimental strategy for analysis of transcription through a nucleosome by single or multiple complexes of RNA polymerase (RNAP). (*A*) Different nucleosome fates during moderate and active transcription in vivo. Only H2A/H2B histones are displaced/exchanged during moderate Pol II transcription (see *Introduction*). In contrast, all core histones are displaced/exchanged on actively transcribed genes. (*B*) Experimental approach using *E. coli* RNAP. Nucleosomal templates containing -UTP (-U) and -UTP/CTP tracts (step 1) are transcribed after formation of single EC45 [(2), the numerical index indicates the position of active center of RNAP on the template relative to the transcription start site] or two tandem stalled elongation complexes [EC11/EC45 (3)] in the presence of corresponding subsets of NTPs. Then transcription is continued in the presence of all NTPs (4). (*C*) Experimental approach using Pol II. EC45 is formed after preassembly and ligation of EC9 and nucleosome [(1) and (2)]. Then the second EC9 is assembled, ligated to EC45 (3), and transcription is continued (4).

Author contributions: O.I.K. and V.M.S. designed research; O.I.K. and F.-K.H. performed research; O.I.K., F.-K.H., and V.M.S. analyzed data; and V.M.S. wrote the paper.

The authors declare no conflict of interest.

This article is a PNAS Direct Submission.

[1]O.I.K and F.-K.H. contributed equally to this work.

[2]To whom correspondence should be addressed. E-mail: studitvm@umdnj.edu.

This article contains supporting information online at www.pnas.org/lookup/suppl/doi:10.1073/pnas.1001148107/-/DCSupplemental.

intermediate(s) mediating nucleosome survival during transcription are disrupted when RNA polymerase encounters previously transcribed subnucleosomes (hexasomes). Thus the efficiencies of nucleosome survival and transcription critically depend on the density of transcribing complexes of RNA polymerase.

## Results

**Experimental Approach.** The primary experimental approach for analysis of the collision between single and multiple complexes of RNAP and nucleosomes is shown in Fig. 1 *B* and *C*. In our studies we used templates that contain uniquely positioned mononucleosomes and recapitulate many important aspects of chromatin transcription in vivo, including displacement of histones H2A/H2B and survival of H3/H4 histones during transcription by single complexes of RNA Pol II (18, 19). Mononucleosomes formed on the high-affinity DNA sequences were utilized in this study (20). Nucleosomes are uniquely positioned on these sequences and present a polar barrier to transcription by Pol II (21). In one (nonpermissive) transcriptional orientation, these nucleosomes provide a strong, factor- and salt-insensitive barrier that Pol II encounters after transcription of ~45 bp in the nucleosomes. In the opposite (permissive) orientation, nucleosomes form a weaker, more diffuse barrier that is largely relieved by TFIIS (transcription factor IIS) or FACT (facilitates chromatin transcription) (14, 22). In this study nucleosomes formed on two high-affinity DNA sequences were used: 603 (permissive) and 601 (nonpermissive) (21). As expected, nucleosomes formed on these sequences are uniquely positioned, and nucleosome preparations usually contain less than 5% of contaminating histone-free DNA (Fig. S1).

Some experiments described below require multiple rounds of initiation from the same template; this is extremely difficult to achieve using Pol II. At the same time, all general aspects of the Pol II-type mechanism of transcription through chromatin are recapitulated by *Escherichia coli* RNAP (18, 23) but not by other previously analyzed RNAPs (21, 24, 25). Therefore the majority of the experiments were conducted using *E. coli* RNAP and yeast Pol II.

One or two tandem complexes of transcribing *E. coli* RNAP were stalled (26) upstream of the mononucleosomes. The first (leading) RNAP complex is stalled after forming 45-nt transcript (EC45) on a −U sequence (Fig. 1*B*). Then the enzyme is immobilized though a His-tag, NTPs are removed, and the second (trailing) RNAP complex is stalled on a −U/C sequence upstream of the leading complex forming EC11. As a result, identical nucleosomal templates contain either a single (EC45) or two tandem complexes of RNAP (EC11/EC45) upstream of the nucleosome. To conduct transcription by Pol II, transcript elongation complexes were assembled from purified yeast Pol II, synthetic DNA oligonucleotides, and short RNAs. Then a single EC9 was immobilized through a His-tag on Pol II, ligated upstream of DNA fragments bearing single nucleosomes, and extended to form EC45 [Fig. 1*C* (19)]. The second Pol II EC9 was then ligated upstream of the EC45, and transcript elongation was continued in the presence of all unlabeled NTPs. The efficiency of transcription and the fate of nucleosomes were analyzed.

**The Nucleosomal Barrier Is Relieved by Two Tandem Transcribing Complexes of RNA Polymerase.** To analyze the effect of the second RNAP complex on the nucleosomal barrier to transcription, EC45 (produced by the leading *E. coli* RNAP complex) was pulse-labeled, followed by a rinsing step and formation of second, unlabeled EC on the same template. In this case the effect of the trailing RNAP complex positioned immediately behind the leading complex on the rate and the efficiency of transcription through the nucleosomes was studied.

As expected, transcription of permissive 603 and nonpermissive 601 nucleosomes by a single *E. coli* RNAP complex results in formation of barriers having very different strengths (Fig. 2 *A* and *B* and Fig. S2). Thus RNAP can overcome the permissive (603) and nonpermissive (601) nucleosomal barriers with ~90% and 40% efficiencies, respectively, at 300 mM KCl. These data are very similar with the results of transcription of these templates by both yeast and human Pol II (21), and they further establish *E. coli* RNAP as an appropriate model for analysis of Pol II-type mechanism of transcription through chromatin.

Encounter of the same nucleosomes by two tandem complexes of RNAP results in much more efficient overcoming of the barrier at all concentrations of KCl, and an increase of the yield of run-off transcripts (Fig. 2 *A* and *B* and Fig. S2). The magnitude of stimulation is higher in the case of the 601 template (high barrier) as compared with the 603 template at all ionic strengths. Remarkably, even the high 601 nucleosomal barrier is almost completely relieved during transcription by two complexes of RNAP (Fig. 2 *A* and *B*). Previously we have shown that the high 601 nucleosomal barrier is poorly relieved by the elongation factors TFIIS and FACT (21) or by removal of histone "tails" (27). Thus the stimulating effect of the second complex of RNAP on transcription through chromatin is stronger than the effect of previously characterized elongation factors and histone tail removal.

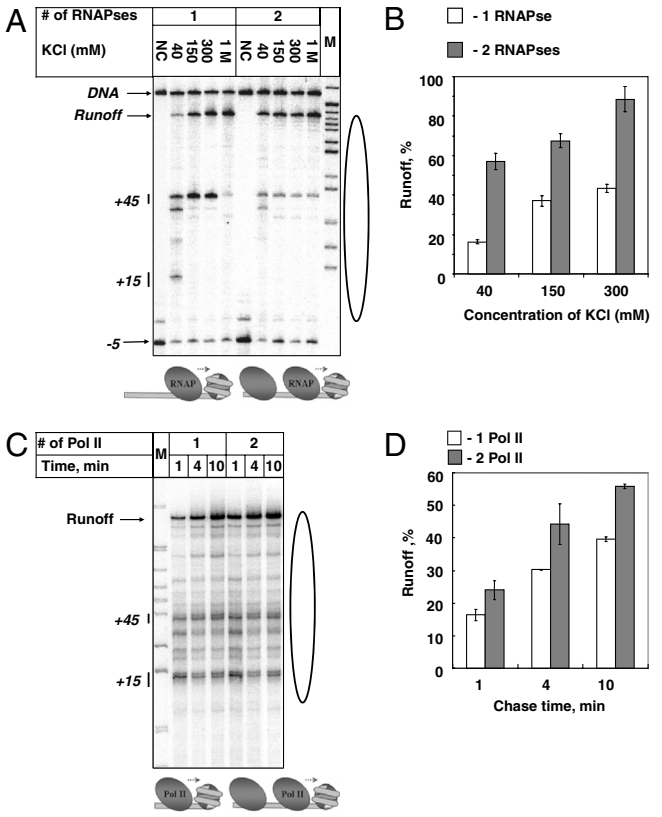


**Fig. 2.** Two complexes of RNAP cooperate to relieve the nucleosomal barrier to transcription in vitro. (*A*) Two complexes of *E. coli* RNAP cooperate to relieve the high (601) nucleosomal barrier to transcription. In all lanes except the first lane (no chase, NC) in each set the initial pulse-labeled 45-mer RNA in EC45 was extended at the indicated concentrations of KCl by a single or two tandem ECs and analyzed by a denaturing PAGE. Position of the nucleosome is indicated by the oval. Positions of the labeled transcripts and DNA are indicated. M—end-labeled pBR322-*Msp*I digest. Note that only two tandem ECs can efficiently overcome the barrier. (*B*). The amounts of the runoff transcripts were quantified using a PhosphorImager and plotted as fraction of the runoff produced at 1 M KCl. Error bars indicate standard deviations; all conditions were tested at least three times. (*C*) Two complexes of Pol II cooperate to relieve the nucleosomal barrier to transcription. Single or two tandem Pol II ECs were assembled as described in Fig. 1*C*. The templates were transcribed at 150 mM KCl for 1, 4, or 10 min. (*D*) Quantitation of the runoff transcripts.

Time courses of transcription of 601 nucleosomes by single or two tandem complexes of RNAP at 40 mM KCl (Fig. S3) indicate that all major nucleosome-specific pauses localized in the region extending from +10 to +45 bp in the nucleosome are strongly affected by the presence of the trailing RNAP complex (Fig. S3*A*). Nucleosome-specific pauses at the positions +10 and +20 are almost entirely eliminated and are very similar with the transient pauses formed during transcription of histone-free DNA. The stronger pausing at the positions +40 and +45 is also considerably diminished. The decrease in the pausing efficiency results in a considerable increase in the yield of runoff transcripts (Fig. S3*B*). The data indicate that the trailing RNAP complex strongly increases the efficiency and the rate of transcription by the leading complex through the nucleosomal barrier. Furthermore, the efficiency of transcription through the 601 nucleosome forming a strong barrier for Pol II approaches the efficiency of transcription characteristic for histone-free DNA (Fig. S3*B*). Although duration of nucleosome-specific pauses is strongly decreased, the nucleosome-specific component is not entirely eliminated, suggesting that the histone octamer remains associated with nucleosomal DNA during transcription by two RNAPses. The results obtained using *E. coli* RNAP and yeast Pol II (Fig. 2 *C* and *D*) are very similar. In both cases the presence of a second enzyme complex results in increased traversal through the nucleosome, and partial relief of all nucleosome-specific pauses.

Efficient transcription through various roadblocks (such as site-specific DNA-binding proteins) and sequence-specific pausing sites by two closely spaced, tandem complexes of RNAP has been described previously (26, 28). It has been shown that the trailing RNAP prevents pausing and arrest of the leading complex of the enzyme by facilitating forward translocation of the leading ECs, most likely by preventing backtracking of the enzyme (26, 28). It has been shown that arrest of Pol II in a nucleosome involves backtracking of the elongation complex over considerable distances along nucleosomal DNA and that blocking of backtracking results in partial relief of nucleosome-induced arrest (22, 29). Therefore most likely the trailing RNAP facilitates transcription through a nucleosome by preventing backtracking of the leading complex of the enzyme.

**Nucleosomes Are Efficiently Converted to Hexasomes by Two Tandem Transcribing Complexes of RNA Polymerase.** Nucleosome displacement observed on highly active genes can occur by at least two mechanisms [Figs. 3*A* and 4*A* (4)]. The first mechanism requires an encounter of the same nucleosome by two tandem complexes of RNAP (Fig. 3*A*). In this case, the trailing complex may prevent DNA rebinding to the octamer behind the leading Pol II complex and thus interfere with nucleosome survival (4). Alternatively, the second Pol II complex could displace the hexasome formed behind the first transcribing Pol II complex (Fig. 4*A*); the latter mechanism does not require nucleosome encounter by two complexes of Pol II at the same time.

To evaluate the first mechanism, DNA-labeled nonpermissive 601 nucleosomes were transcribed by a single or two closely spaced complexes of *E. coli* RNAP at various concentrations of KCl, and the fates of nucleosomes on transcription were analyzed by native PAGE (Fig. 3*B*). As expected, the EC formed by two complexes of RNAP are more efficiently converted into the products of transcription (histone-free DNA and hexasomes missing one H2A/H2B dimer) than by the single complexes. Transcription by a single or two tandem complexes of RNAP results in displacement of a similar fraction of DNA (~50–60%) from the transcribed nucleosomal templates (Fig. 3 *C* and *D*). Transcription by a single or two tandem complexes of Pol II also resulted in displacement of a similar fraction of DNA (~15–25%) from the transcribed nucleosomes in both cases (Fig. 3 *E* and *F*). Thus, surprisingly, in all cases a considerable fraction of hexasomes (from 40% to 85%) survives during transcription by a single and by two tandem, closely spaced complexes of RNAP. Note that yeast Pol II cannot complete traversal through the 601 template (21); therefore the 603 template was utilized.

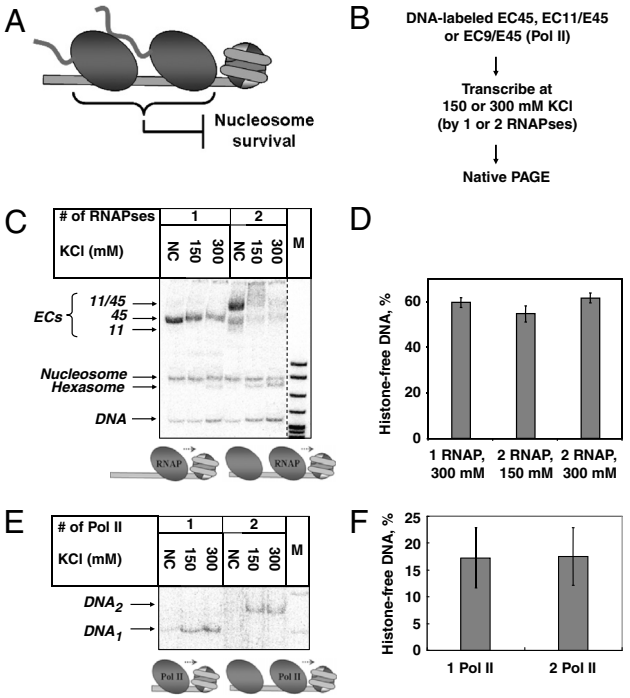


**Fig. 3.** Nucleosomes are converted to hexasomes by two tandem complexes of transcribing RNAP. To evaluate a possibility that tandem, closely spaced Pol II complexes prevent nucleosome survival during transcription (*A*), DNA-labeled nucleosomes were transcribed by single (EC45) or tandem complexes of *E. coli* RNAP (EC11/EC45) or Pol II (EC9/EC45) at various concentrations of KCl, and the products of the reaction were analyzed by native PAGE (*B*). (*C*) Transcription of nonpermissive 601 nucleosomes by *E. coli* RNAP. Positions of histone-free DNA and various DNA-protein complexes are indicated. (*D*) Quantitation of the amounts of histone-free DNA produced after transcription by one or two complexes of RNAP (plotted as fraction of all templates transcribed to completion). The amounts are similar, suggesting that hexasomes can survive transcription by two closely spaced tandem RNAP complexes. Note that only a small fraction of templates was transcribed to completion by one RNAP complex at 150 mM KCl; therefore, in this case, the fraction of displaced DNA cannot be reliably quantified. (*E*) Transcription of permissive 603 nucleosomes by Pol II. Histone-free DNA displaced from the octamer by single ($DNA_1$) and tandem transcribing complexes of Pol II ($DNA_2$) is shown. (*F*) Quantitation of histone-free DNA displaced by Pol II at 150 mM KCl.

How can DNA-bound histones survive transcription by two tandem, closely spaced complexes of Pol II? Previously we have shown that during transcription by one Pol II complex, nucleosome survival depends on efficient formation of a "Ø-loop," a small intranucleosomal DNA loop containing transcribing Pol II at the position +49 bp in the nucleosome [Fig. S4 (18)]. In particular, nucleosome survival depends on Pol II-histone interactions in the Ø-loop and on the upstream DNA-histone interactions (18). Tandem Pol II complexes that encounter a roadblock (e.g., a nucleosome) tend to be spaced by only 26–32 bp (28). If such closely spaced complexes of Pol II have similar rotational orientations on nucleosomal DNA, the DNA-histone interactions behind the leading Pol II complex could be replaced by the trailing Pol II-histone interactions stabilizing the Ø-loop (Fig. S4, Inset). In this case the leading Pol II complex could continue transcription without displacement of histones H3/H4.

**Single Complex of RNA Polymerase Can Displace DNA-Bound Histone Hexamer Formed During the Previous Round of Transcription.** Since two tandem complexes of Pol II allow survival of the histone hexamer after transcription (Fig. 3), next we evaluated the model

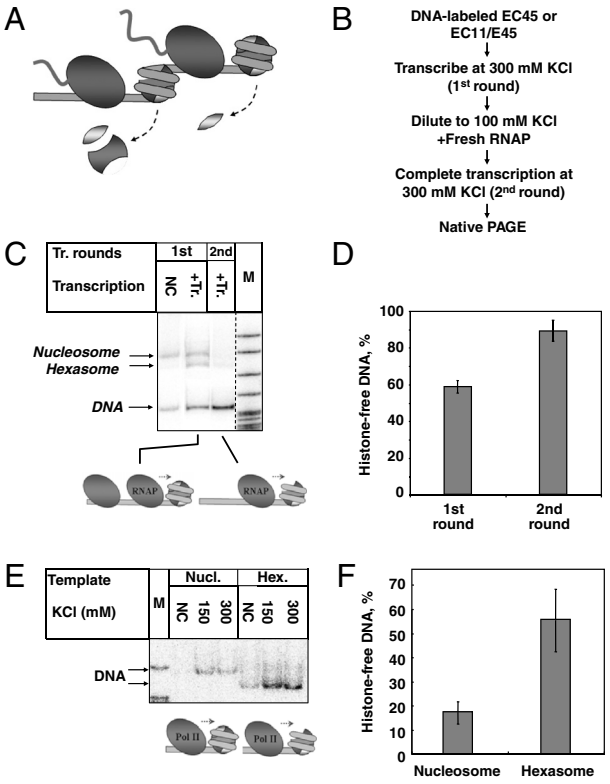


**Fig. 4.** DNA-bound histone hexamer is displaced by transcribing RNAP. To evaluate a possibility that Pol II displaces the hexasome formed as a result of previous round of transcription (*A*), single-round or multiple-round transcription of the nonpermissive 601 nucleosomal template by *E. coli* RNAP were conducted (*B*), and DNA-labeled templates were analyzed by a native PAGE (*C*). (*D*) Quantitation of the amounts of histone-free DNA produced after single or multiple rounds of transcription. Multiple rounds of transcription result in strong increase in the amount of histone-free DNA, suggesting that histone hexamer is evicted from DNA. (*E*) Removal of H2A/H2B dimer facilitates histone displacement during Pol II transcription. A shortened template was utilized to prevent binding of the promoter-distal H2A/H2B dimer. Nucleosomes or hexasomes were formed on the full-length or the shortened 603 templates, respectively, and transcribed by a single Pol II complex at different KCl concentrations. (*F*) Quantitation of histone-free DNA displaced by Pol II.

involving sequential encounter of multiple complexes of Pol II with the same nucleosome (Fig. 4*A*). In this case the trailing complex of Pol II encounters the hexasome formed after transcription by leading Pol II complex.

Single-round transcription through the 601 nucleosome by *E. coli* RNAP was conducted at 300 mM KCl to form the hexasome (Fig. 4 *B* and *C*). Then reinitiation of transcription was allowed after dilution of KCl to 100 mM and adding fresh RNAP, and an additional round of transcription was completed at 300 mM KCl. After the second round of transcription the fraction of displaced DNA is increased from ~60% to >90% (Fig. 4*D*), indicating that all core histones were displaced from DNA on the vast majority of the templates (~90%).

Histone displacement from the hexasomes during the second round of transcription could occur due to (*I*) an insufficient number of DNA-histone interactions present in the hexasomes and (*ii*) conformational changes in the nucleosome structure introduced during the first round of transcription (besides displacement of the H2A/H2B dimer). To discriminate between these possibilities, hexasomes were formed on the 601 sequence that was shortened by 38 bp from the promoter-distal DNA end (Fig. S5*A*). Such hexasome templates are transcribed by Pol II more efficiently than intact nucleosomes (18). Preformed nucleosomes and hexasomes were ligated to T7A1 promoter (*E. coli* RNAP) or to preassembled EC9 (Pol II), and the templates were transcribed at various concentrations of KCl. The predominant product of transcription through the hexasomes by *E. coli* RNAP is DNA (>80%, Fig. S5*B*), as compared with ~60% DNA formed after transcription of intact nucleosomes. A similar result was obtained using yeast Pol II (Fig. 4 *E* and *F*). In this case, 17.5% DNA and 56% DNA were displaced after transcription by single complex of the enzyme through nucleosomes and hexasomes, respectively. Thus transcription causes eviction of all core histones from hexasomes formed after a previous round of transcription and from nontranscribed hexasomes preformed on the template. Evidently, previous transcription is not required for histone displacement from the hexasomes.

Most likely, the intermediates formed during transcription through hexasomes are less stable than the intermediates formed on the nucleosome. Indeed, each histone H2A/H2B dimer stabilizes ~35-bp region of nucleosomal DNA (30). As Pol II enters into a nucleosome (Fig. 5*A*), it initially (before Pol II reaches the position +49) disrupts DNA-histone interactions behind the transcribing complex (18). As a result, a Pol II-nucleosome +45 complex missing promoter-distal H2A/H2B is stabilized by a small number of DNA-histone interactions (Fig. 5*A*, complex 2′), and the entire histone octamer could spontaneously dissociate into solution.

## Discussion

In summary, the fate of nucleosomes on transcription and the efficiency of transcription through chromatin depend on template

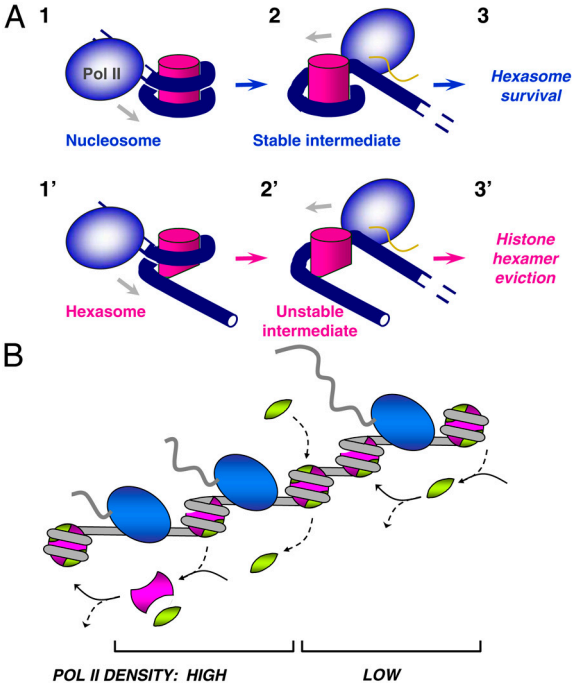


**Fig. 5.** Proximity of Pol II complexes to each other dictates the fate of nucleosomes on transcription. (*A*). A proposed mechanism of histone hexamer eviction by transcribing Pol II. Pathway 1 → 2 → 3: The mechanism of nucleosome survival during transcription (18). As Pol II approaches (step 1) and enters in a nucleosome (2), it uncoils the upstream DNA. However, the downstream DNA remains associated with the octamer forming a stable intermediate that allows nucleosome survival (3). In contrast, during Pol II transcription through a hexasomes missing one H2A/H2B dimer (1′), an unstable intermediate with a smaller number of DNA-histone contacts is formed (2′), resulting in eviction of the histone hexamer from DNA (3′). (*B*) A high density of transcribing Pol II complexes can result in histone eviction from transcribed genes. At a low Pol II density transcription is accompanied by transient displacement/exchange of H2A/H2B dimer(s); the nucleosome structure is recovered before arrival of the next Pol II complex. At a higher density Pol II complexes encounter hexasomes that are missing H2A/H2B dimer(s); therefore, all core histones are evicted and exchanged.

**Table 1. The fate of histones on transcription and the efficiency of transcription through chromatin**

| Encounter | Height of the nucleosomal barrier | Histone displacement |
|---|---|---|
| Pol II-nucleosome | +++ | H2A/H2B |
| $(Pol II)_2$-nucleosome* | + | H2A/H2B |
| Pol II-hexasome† | + | All core histones |

*Closely spaced, tandem Pol II complexes encounter a nucleosome.
†Pol II complex encounters previously transcribed hexasome.

composition and on the number of complexes of Pol II that encounter nucleosomes (Table 1). Two tandem complexes of RNA polymerase overcome the nucleosomal barrier more efficiently than one complex. When the trailing complex closely follows the leading transcribing complex of RNAP and both complexes encounter the same nucleosome, the rate and efficiency of transcription through the barrier are increased (Fig. 2 and Figs. S2 and S3), one H2A/H2B dimer is displaced, and the nucleosome is converted into a hexasome (Fig. 3). When a nucleosome is sequentially encountered by two single complexes of Pol II, the first complex encounters a high nucleosomal barrier and converts the nucleosome into a hexasome. The second complex of Pol II efficiently proceeds through the resulting hexasome and displaces all core histones (Fig. 4).

Our *in vitro* experiments suggest that the histone hexasome (nucleosome missing one H2A/H2B dimer) left behind transcribing Pol II can be disrupted by the next transcribing complex (see *Results* and Fig. 5*A*). However, H2A/H2B histones on moderately transcribed genes are not displaced from DNA, but rapidly exchanged (see *Introduction*), suggesting that H2A/H2B dimers rebind to transcribed hexasomes at a high rate in vivo. In contrast, H3/H4 are displaced/exchanged only during intense transcription in vivo (see *Introduction*). Taken together, these observations suggest the following scenario for Pol II transcription in vivo (Fig. 5*B*). Low-level to moderate-level transcription by single, widely spaced complexes of Pol II is characterized by displacement/exchange of only H2A/H2B dimer(s). The dimers rebind to the hexasomes before the next Pol II complex arrives; therefore, each complex encounters complete nucleosomes, and there is no functional cooperation between the complexes. This is consistent with the observation that the loss of certain histone chaperones that could function to restore H2A and H2B also leads to loss of nucleosomes within transcription units (2).

As the transcription rate is increased, the distance between transcribing Pol II complexes becomes shorter, and trailing complexes of Pol II may encounter the hexasome formed after the previous round of transcription, before the H2A/H2B dimer rebinds to the hexasome (Fig. 5*B*). In this case all core histones are displaced from DNA (11–13). Thus the model suggested by our experiments explains both the lack of exchange of H3/H4 histones at moderate transcription and their exchange at actively transcribed genes. Furthermore, the model provides an explanation for the gradual dependence of the rates of histone displacement/exchange on the efficiency of transcription (8, 11–13). Indeed, the cooperating complexes of Pol II do not have to invade the same nucleosome and the extent of histone displacement would be inversely proportional to the average distance between transcribing Pol II complexes. It should be noted that on some inducible genes partial transcription-independent loss of all core histones has been observed (31, 32).

Recently it has been discovered that stalled Pol II is observed immediately downstream of thousands of human and *Drosophila* promoters, close to the +1 nucleosome (33–35). The majority of these genes are inactive in the sense that no transcripts longer than ~100 nt are made. These observations suggest that the earliest stages of transcript elongation can constitute a major step in gene regulation in vivo. Given the typical location for these stalled polymerases [the active center is localized 30–50 bp downstream of the start site (36)], it is reasonable to suggest that induction of transcription on such genes would lead to accumulation of two closely spaced Pol II elongation complexes upstream of the initial nucleosome. Such tandem polymerases (Pol II "dimer") would overcome the +1 nucleosome or other elongation blocks (e.g., DNA-bound proteins) more efficiently than a single Pol II complex and would displace only H2A/H2B histones from DNA. We suggest that the Pol II dimer could serve as a "pioneering" Pol II. Indeed, nucleosomes present a strong barrier to single complexes of transcribing Pol II in vitro (19, 21, 37) and perhaps on inactive genes in vivo (35, 38). According to this concept, after gene activation the pioneering Pol II dimer would be able to overcome the +1 nucleosomal barrier and efficiently transcribe the entire gene. During the initial round of transcription, chromatin structure is perturbed and histones become more accessible to covalent modifications characteristic for transcribed genes (39, 40). Such histone modifications could then serve as a "memory" (41, 42) and facilitate subsequent rounds of transcription.

## Materials and Methods

**Templates and Proteins.** Hexahistidine-tagged *E. coli* RNAP, yeast Pol II, and core histones were purified as described (18). The 603 and 601 templates for Pol II and *E. coli* RNAP were described previously (18, 21). Nucleosomes and the hexasomes were reconstituted by octamer exchange at 1 M NaCl or by dialysis from 2 M NaCl, respectively (18).

**Transcription and Analysis of Nucleosomes and Subnucleosomes.** *E. coli* RNAP: Formation of various leading elongation complexes (EC11 and EC 45 containing 11-mer and 45-mer RNA, respectively) and transcription were conducted as described (18). In short, EC11 was formed on preassembled nucleosomal templates. In experiments with labeled RNA, EC45 was pulse-labeled in the presence of [$\alpha$-$^{32}$P]-labeled GTP. Then EC45 was immobilized on Ni-NTA-agarose, purified from NTPs, and eluted into solution. Then trailing EC11 was assembled using a very similar protocol (18) and transcribed in the presence of all NTPs.

Pol II: Transcription by Pol II was performed as described (18), with minor modifications. EC9 complexes were preformed on DNA templates containing 9-nt single-stranded DNA sticky ends downstream (trailing EC), or upstream and downstream (leading EC) of the ECs. The ECs were separately immobilized on Ni-NTA-agarose, washed, and eluted into solution. The leading EC Pol II was advanced to form EC45 in the presence of [$\alpha$-$^{32}$P]-labeled GTP. Then the immobilized ECs were ligated to each other or mock-ligated, and transcribed in the presence of all NTPs.

Labeled RNA was purified and separated by denaturing PAGE (18). DNA-labeled complexes were analyzed by native PAGE (21).

Labeled run-off transcripts were quantified using a PhosphorImager as described (21). Bands in native gels containing DNA-labeled complexes were quantified and corrected for loading. The fractions of fully transcribed templates were determined by quantifying active elongation complexes and the products of transcription (DNA and hexasomes) having distinct mobilities in the gel before and after transcription (19). The yields of the products of transcription were calculated and normalized for the amounts of fully transcribed templates.

**ACKNOWLEDGMENTS.** We thank John Widom for plasmids containing the nucleosome positioning sequences and Don Luse for suggestions and critical reading of the manuscript. This work was supported by NIH GM58650 Grant to V.M.S.

1. Martens JA, Wu PY, Winston F (2005) Regulation of an intergenic transcript controls adjacent gene transcription in *Saccharomyces cerevisiae*. *Genes Dev* 19:2695–2704.
2. Cheung V, et al. (2008) Chromatin- and transcription-related factors repress transcription from within coding regions throughout the *Saccharomyces cerevisiae* genome. *PLoS Biol* 6:e277.
3. Thiriet C, Hayes JJ (2006) Histone dynamics during transcription: exchange of H2A/H2B dimers and H3/H4 tetramers during pol II elongation. *Results Probl Cell Differ* 41:77–90.
4. Kulaeva OI, Gaykalova DA, Studitsky VM (2007) Transcription through chromatin by RNA polymerase II: Histone displacement and exchange. *Mutat Res* 618:116–129.

5. Wirbelauer C, Bell O, Schubeler D (2005) Variant histone H33 is deposited at sites of nucleosomal displacement throughout transcribed genes while active histone modifications show a promoter-proximal bias. *Genes Dev* 19:1761–1766.
6. Schwartz BE, Ahmad K (2005) Transcriptional activation triggers deposition and removal of the histone variant H3.3. *Genes Dev* 19:804–814.
7. Thiriet C, Hayes JJ (2005) Replication-independent core histone dynamics at transcriptionally active loci *in vivo*. *Genes Dev* 19:677–682.
8. Dion MF, et al. (2007) Dynamics of replication-independent histone turnover in budding yeast. *Science* 315:1405–1408.
9. Rufiange A, et al. (2007) Genome-wide replication-independent histone H3 exchange occurs predominantly at promoters and implicates H3 K56 acetylation and Asf1. *Mol Cell* 27:393–405.
10. Jamai A, Imoberdorf RM, Strubin M (2007) Continuous histone H2B and transcription-dependent histone H3 exchange in yeast cells outside of replication. *Mol Cell* 25:345–355.
11. Kristjuhan A, Svejstrup JQ (2004) Evidence for distinct mechanisms facilitating transcript elongation through chromatin *in vivo*. *EMBO J* 23:4243–4252.
12. Lee CK, et al. (2004) Evidence for nucleosome depletion at active regulatory regions genome-wide. *Nat Genet* 36:900–905.
13. Schwabish MA, Struhl K (2004) Evidence for eviction and rapid deposition of histones upon transcriptional elongation by RNA polymerase II. *Mol Cell Biol* 24:10111–10117.
14. Kireeva ML, et al. (2002) Nucleosome remodeling induced by RNA polymerase II. Loss of the H2A/H2B dimer during transcription. *Mol Cell* 9:541–552.
15. Belotserkovskaya R, et al. (2003) FACT facilitates transcription-dependent nucleosome alteration. *Science* 301:1090–1093.
16. Angelov D, et al. (2006) Nucleolin is a histone chaperone with FACT-like activity and assists remodeling of nucleosomes. *EMBO J* 25:1669–1679.
17. Kimura H, Cook PR (2001) Kinetics of core histones in living human cells: little exchange of H3 and H4 and some rapid exchange of H2B. *J Cell Biol* 153:1341–1353.
18. Kulaeva OI, et al. (2009) Mechanism of chromatin remodeling and recovery during passage of RNA polymerase II. *Nat Struct Mol Biol* 16:1272–1278.
19. Kireeva ML, et al. (2002) Nucleosome remodeling induced by RNA polymerase II: Loss of the H2A/H2B dimer during transcription. *Mol Cell* 9:541–552.
20. Thastrom A, et al. (1999) Sequence motifs and free energies of selected natural and non-natural nucleosome positioning DNA sequences. *J Mol Biol* 288:213–229.
21. Bondarenko VA, et al. (2006) Nucleosomes can form a polar barrier to transcript elongation by RNA polymerase II. *Mol Cell* 24:469–479.
22. Kireeva ML, et al. (2005) Nature of the nucleosomal barrier to RNA polymerase II. *Mol Cell* 18:97–108.
23. Walter W, Kireeva ML, Studitsky VM, Kashlev M (2003) Bacterial polymerase and yeast polymerase II use similar mechanisms for transcription through nucleosomes. *J Biol Chem* 278:36148–36156.
24. Studitsky VM, Clark DJ, Felsenfeld G (1994) A histone octamer can step around a transcribing polymerase without leaving the template. *Cell* 76:371–382.
25. Studitsky VM, Kassavetis GA, Geiduschek EP, Felsenfeld G (1997) Mechanism of transcription through the nucleosome by eukaryotic RNA polymerase. *Science* 278:1960–1963.
26. Epshtein V, Nudler E (2003) Cooperation between RNA polymerase molecules in transcription elongation. *Science* 300:801–805.
27. Ujvari A, et al. (2008) Histone N-terminal tails interfere with nucleosome traversal by RNA polymerase II. *J Biol Chem* 283:32236–32243.
28. Saeki H, Svejstrup JQ (2009) Stability, flexibility, and dynamic interactions of colliding RNA polymerase II elongation complexes. *Mol Cell* 35:191–205.
29. Hodges C, et al. (2009) Nucleosomal fluctuations govern the transcription dynamics of RNA polymerase II. *Science* 325:626–628.
30. Luger K, et al. (1997) Crystal structure of the nucleosome core particle at 28 A resolution. *Nature* 389:251–260.
31. Zhao J, Herrera-Diaz J, Gross DS (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.
32. Petesch SJ, Lis JT (2008) Rapid, transcription-independent loss of nucleosomes over a large chromatin domain at *Hsp70* loci. *Cell* 134:74–84.
33. Guenther MG, et al. (2007) A chromatin landmark and transcription initiation at most promoters in human cells. *Cell* 130:77–88.
34. Zeitlinger J, et al. (2007) RNA polymerase stalling at developmental control genes in the *Drosophila melanogaster* embryo. *Nat Genet* 39:1512–1516.
35. Mavrich TN, et al. (2008) Nucleosome organization in the *Drosophila* genome. *Nature* 453:358–362.
36. Muse GW, et al. (2007) RNA polymerase is poised for activation across the genome. *Nat Genet* 39:1507–1511.
37. Izban MG, Luse DS (1991) Transcription on nucleosomal templates by RNA polymerase II *in vitro*: Inhibition of elongation with enhancement of sequence-specific pausing. *Genes Dev* 5:683–696.
38. Sullivan EK, et al. (2001) Transcriptional activation domains of human heat shock factor 1 recruit human SWI/SNF. *Mol Cell Biol* 21:5826–5837.
39. Zippo A, et al. (2009) Histone crosstalk between H3S10ph and H4K16ac generates a histone code that mediates transcription elongation. *Cell* 138:1122–1136.
40. Wang Z, et al. (2009) Genome-wide mapping of HATs and HDACs reveals distinct functions in active and inactive genes. *Cell* 138:1019–1031.
41. Carrozza MJ, et al. (2005) Histone H3 methylation by Set2 directs deacetylation of coding regions by Rpd3S to suppress spurious intragenic transcription. *Cell* 123:581–592.
42. Ng HH, Robert F, Young RA, Struhl K (2003) Targeted recruitment of Set1 histone methylase by elongating Pol II provides a localized mark and memory of recent transcriptional activity. *Mol Cell* 11:709–719.