Mechanism of Chromatin Remodeling and Recovery during Passage of RNA Polymerase II

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Supplementary Figure 1 Asymmetric distribution of HA sequences. Top: 601, 603 and 605 sequences are aligned according to their transcriptional orientations (arrows). The extent of identity of the left (L) and right (R) sequences with the HA consensus (red) is shown (%). The nucleosomal dyad is indicated. Bottom: Modified 603R DNA sequences [603R-L, -R, -R3 and -R(2-3)] and the position of Pol II arrest (red arrow) are indicated.
Supplementary Figure 2

**a**

- Yeast EC
- Immobilization
- Ligation
- EC-119
- EC-83
- Elution, +all NTPs
- Transcript elongation

**b**

- E. coli T7A1
- 603
- Initiation/+AUC + ATP + GTP
- EC-39
- +CTP
- EC-5
- +UTP
- EC+41 or EC+49

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Supplementary Figure 2 The experimental system. (a) The primary experimental approach for Pol II transcription. The elongation complex EC-119 was assembled using yeast Pol II, DNA oligonucleotides and short RNA primers (the numerical index indicates the position of the active center on the template relative to promoter-proximal nucleosomal DNA boundary). The complex was immobilized on Ni-NTA-agarose beads and ligated to DNA or nucleosomal templates containing 603 or 603R sequences. In some experiments Pol II was advanced in the presence of a subset of NTPs and [α-32P] GTP to produce RNA-pulse-labeled EC-83 complex. The immobilized complexes were washed, eluted, and transcription was resumed by addition of all unlabeled NTPs. (b) The experimental approach for stalling of the E. coli ECs at unique positions on the 603 template. The sequences of the two 603 templates (603-42 and 603-49) allow progression to and stalling at -39, -5 (both templates), +41 or +49 positions upon addition of different partial combinations of NTPs (see Methods).
Supplementary Figure 3 Charge distribution on the contacting surfaces of Pol II and histones in the modeled EC+39. The contacting surfaces of Pol II within the EC+39 (on the right, the histone octamer is not shown) and the histone octamer (on the left) are shown. The darkest blue and the darkest red denote a potential of 82.3 kT/e and -82.3 kT/e, respectively. The electrostatic surfaces that have the opposite charges and are in close proximity in the EC+39 are indicated by rectangles. DNA (left) and DNA path in the original nucleosome (right) are shown in yellow. The positions +39 and +49 on nucleosomal DNA, and some of the histone “tails” are indicated. Pol II and nucleosome structures (PDB IDs 1aoi and 1y1w, see refs. 13, 15) were used.
**a** RNA-pulse-labeled EC−39 +CTP → EC−5 +UTP → EC+41 or EC+49 +NTPs → Denaturing PAGE

EC:
-39 −5 +41 +49 +NTPs

Run-off →

+49 +42

−5 →

−39 → M

**b** RNA pulse-labeled EC+49 or EC+41

-/+GreB -/+ NTPs 1M KCl → Denaturing PAGE

GreB: − 1 1

Chase: − − +

EC+49

Run-off →

+49 →

+13 +4

M

EC+41

RNA 3′-end

GreB

+42 +41

M

Supplementary Figure 4
Supplementary Figure 4  Active ECs can be stalled at unique positions on nucleosomal DNA. (a) Analysis of ECs formed by *E. coli* RNAP on permissive 603 nucleosomes. Top: The experimental approach. Bottom: Analysis of pulse-labeled RNA by denaturing PAGE. The ECs contain extendable RNA. The chase experiments were conducted at 1 M KCl to disrupt the nucleosome and facilitate transcription. (b) RNAP is stalled at the positions +41 or +49 on the 603 templates. Top: The experimental approach for mapping of positions of the active center of RNAP on DNA. The extent of backtracking was measured using GreB factor. Bottom: Analysis of pulse-labeled RNA by denaturing PAGE. Left: The majority of the EC+49 complexes are not cleaved in the presence of GreB. The GreB preparation was active because cleavage of shorter RNA molecules was observed (bottom of the gel). Right: 1 to 2 nt of RNA is cleaved in EC stalled at +42 indicating that RNAP backtracks by 1-2 bp to form EC+41 complex.
Supplementary Figure 5 Accessibilities of the intermediates formed during transcription through a nucleosome by Pol II to restriction enzymes. (a) Sensitivities of the EC-5, EC+41 and EC+49 complexes (1, 2 and 3) to restriction enzymes: analysis by native PAGE. The top of the gel is shown. The complexes having higher mobilities in the gel (the intense bands) contain Pol II molecules arrested at multiple positions 10-20 bp downstream from the start-site, before reaching the nucleosome. The mobility of EC+49 (but not EC+41) complexes is changed after digestion with Styl (dots), indicating that the Styl site is more sensitive in EC+49 than in EC+41. (b) The bottom part of the gel: protein-free DNA present in the sample and released from the DNA-protein complexes after digestion.
Supplementary Figure 6. The experimental approach: removal of promoter-proximal or promoter-distal H2A/H2B dimer. (a) Characterization of the hexasomes formed on the 110-bp (-P dimer) DNA fragment of the permissive 603 template. Hexasomes (Hex.) were reconstituted in the presence of all core histones and analyzed by native PAGE. Hexasome preparations contain small amounts of contaminating H3/H4 tetrasomes (Tetr.) and histone-free DNA. (b) The nucleosomes or subnucleosomes formed on 603 DNA fragments having different lengths, were ligated to the Pol II EC containing 9-mer RNA through the TspRI site and transcribed.
**Supplementary Figure 7.** Proposed Pol II-type and Pol III-type mechanisms of transcription through a nucleosome. As RNAPses enter the nucleosome (1), they partially displace DNA from the surface of the histone octamer (2 and 2'). However, more extended downstream DNA region is displaced during transcription by Pol III-type mechanism $^{10}$ than during transcription by Pol II-type mechanism (Fig. 3e). Therefore we propose that formation of the Ø-loop by Pol II (3) occurs more efficient than by Pol III, and the latter enzyme tends to form larger DNA loops (3'). Formation of the loops induces disruption of DNA-histone interactions in front of RNA polymerases (4 and 4'), and recovery of the nucleosome at the original position (Pol II, 5) or at an upstream position (Pol III, 5') on DNA.
Supplementary Figure 8
**Supplementary Figure 8.** A model of an intranucleosomal TtRNA-containing DNA Ø-loop. (a) Schematic representation of the structure. (b) A stereo diagram of the complex. The structures of a nucleosome (PDB ID 1aoi, see ref. 13) and *T. thermophilus* RNAP EC (PDB ID 2o5i, see ref. 14) with the active site at the position +39 were merged using the docking approach. The bridge helix, the clamp, the C-terminal coiled coil and the rest of the RNAP molecule are in magenta, cyan, brown and grey, respectively. Other designations as in Figure 2. (c) The structure was rotated by ~90 degrees around the vertical axis.
Supplementary Discussion

Productive elongation complexes are formed at the desired positions on the majority of the templates

RNA analysis (Supplementary Fig. 4a) and footprinting data (Fig. 3b) suggest that the majority (>80%) of RNAP molecules are stalled at the desired position (-39) in the EC-39 complexes. In this case there is quantitative agreement between the data obtained by the two techniques, indicating that both methods detect the same ECs. Quantitative comparison of RNA content of the EC-39 and EC+41/EC+49 complexes (Supplementary Fig. 4a) suggests that the majority (>70%) of RNAP molecules are stalled at the desired positions in all these complexes. This conclusion is confirmed by the observation that the majority of stalled ECs can be extended to produce the run-off transcript (Supplementary Fig. 4b) and therefore remain functionally active.

In summary, quantitative analysis suggests that: (a) The majority (>70%) of RNAP molecules are stalled at the desired positions and remain transcriptionally active in all ECs (EC-39, EC-5, EC+41 and EC+49). (b) RNA analysis and footprinting detect the same ECs.

Structural features of the modeled intranucleosomal Pol II EC+39

The steric constraints revealed during modeling of the EC+39 (Fig. 2) are not sufficiently strict to allow reliable identification of the potential interacting side chains that form Pol II-nucleosome interface in the EC+39. To further evaluate the potential interacting surfaces in the complex, charge distribution on the Pol II-nucleosome interface (PDB IDs 1aoi and 1y1w, see refs. 13, 15) was analyzed (Supplementary Fig. 3). This analysis revealed a strong negative charge on the surface of Pol II in close proximity to positively charged region on the surface of the histone octamer. These regions are located within the clamp core domain of RPB1 subunit of Pol II1 and most likely form electrostatic interactions within the EC+39. The same positively charged region on the surface of the histone octamer interacts with DNA in the original nucleosome; these interactions are disrupted in the EC+39. Therefore the electrostatic Pol II-histone interactions
within the EC+39 may compensate for the DNA-histone interactions that are disrupted during formation of the elongation complex, and thus stabilize the EC+39. Similar interactions may stabilize the EC+49 complex.

If the negatively charged region on the surface of Pol II is important for proper transcription through chromatin, this region is expected to be conserved between *E. coli* RNAP and Pol II that transcribe through chromatin using similar mechanisms. Sequence comparison of the relevant negatively charged regions of the β' (*E. coli* RNAP) and RPB1 (*S. cerevisiae* Pol II) subunits shows 39% sequence identity and 59% sequence similarity. In particular, 5 out of 6 critical negatively charged residues are preserved and one residue is replaced by a polar amino acid. More than 50% of the net negative charge of the conserved region is preserved in *E. coli* RNAP.

Taken together, the data suggest that the negatively charged region on the surface of Pol II could be important for proper transcription through chromatin. Most likely, this region forms electrostatic interactions with the histone octamer in EC+39 and/or EC+49 complexes to compensate for the absence of contacts of the basic histone residues with the phosphate backbone of displaced DNA and thus stabilizes these critical complexes.

**Conserved features of the Pol II-type mechanism of transcription through chromatin**

Although the majority of the data supporting the Pol II-type mechanism of transcription through chromatin were obtained using nucleosomes positioned on high-affinity sequences, the deduced mechanism likely has a general significance. Indeed, the 600-series nucleosomes in permissive orientation recapitulate all transcription properties of nucleosomes formed on low-affinity DNA sequences (2 and data not shown). Moreover, on all studied templates (having either low or high affinities to core histones), the pattern of nucleosome-induced pausing (and therefore the underlying mechanism) is highly conserved. As Pol II approaches the +45 region, pausing is increased, reaching maximum in the +45 region, and then sharply declines2-5, most likely because DNA is uncoiled from the octamer ahead of transcribing Pol II molecule.
The Pol II-type and Pol III-type mechanisms of transcription through chromatin: the structures of the intermediates

Two strikingly distinct pathways of transcription through chromatin (Pol II- and Pol III-type mechanisms) have been described\(^4,6,7\). The signatures of the Pol III-type mechanism (used by bacteriophage SP6 RNAP and yeast Pol III) are a lower nucleosomal barrier to transcription and transfer of the entire histone octamer during transcription. In contrast, the Pol II-type mechanism is characterized by loss or exchange of H2A/H2B dimer(s) during transcription\(^4,8,9\), a stronger barrier to transcription, and survival of the remaining histones at their original position on DNA\(^4\).

The structures of the intermediates formed during transcription through a nucleosome by Pol III-type mechanism were characterized previously\(^10\) (Supplementary Fig. 7). The structures of the EC+41 formed by E. coli RNAP, Pol II and SP6 RNAP are similar: DNA upstream of the EC is uncoiled from the octamer and fully accessible to DNase I and restriction enzymes (\(^10\) and Figs 3 and 4). However during transcription by Pol III-type mechanism DNA is also partially uncoiled from the octamer immediately downstream of the EC+41\(^10\). Thus during early stages of transcription by Pol III-type mechanism a larger octamer surface is exposed to solution (Supplementary Fig. 7). This observation may explain the higher probability of transfer of the histone octamer to DNA immediately upstream of the EC during transcription by Pol III-type mechanism\(^6,11\). Accordingly, Ø-loop-containing complex is formed only on 30% of templates\(^10\). In contrast, the lack of DNA uncoiling in front of Pol II or E. coli RNAP could make formation of the Ø-loop and accompanying displacement of the promoter-distal end of nucleosomal DNA more efficient. Since formation of the Ø-loop allows persistent octamer-DNA interactions, transcription by Pol II is fully compatible with the efficient nucleosome survival at the original position on DNA.
Supplementary Methods

Modeling of Pol II at the position +39 in a nucleosome

The model was built manually using the O program and the structure of nucleosome (PDB ID 1a0i, see ref. 13) to which at the first step the high resolution structure of the bacterial EC (PDB ID 2o5i, see ref. 14) was docked (Supplementary Fig. 8). 50-bp DNA region was displaced from the octamer surface starting from the end of nucleosomal DNA downstream of the EC to allow formation of the Ø-loop. The 30 bp of nucleosomal DNA was also removed at and around the position of the active center of the enzyme (+39), including upstream and downstream sequences that constitute the transcription bubble buried within the RNAP structure (Ø-loop).

The bacterial enzyme revealed no significant steric clashes with the nucleosome. On the next step, the structure of the yeast Pol II was modeled on the nucleosome through superposition of the bacterial RNAP and Pol II backbones. In the model, structural configuration of the nucleic acids in the transcription bubble observed in the experimental structures of the bacterial and eukaryotic ECs remained nearly intact. The structure of the histone core was not modified except for truncation of several N-terminal histone tails facing RNAP to avoid steric hindrance with the enzyme. In the complex with RNAP, these protruding flexible tails may readily adopt drastically distinct conformations as compared to the intact nucleosome structure; therefore modeling of the positions of histone tails is not feasible. The only notable (but still quite subtle) alterations, in which several Pol II protruding structural domains on the surface were slightly rotated as the rigid bodies (by ~35-40°) to avoid close contacts with the neighboring histone fragments, resulted in opening of the Pol II “jaws” and, subsequently, in a slight widening (4-5Å) of the main cavity (Fig. 2). Similar alterations have been observed in previously described Pol II structures. The Figure 2 was prepared using the programs Molscript, Bobscript, and Raster3D.
Analysis of the structural features of the modeled intranucleosomal Pol II EC+39

The molecular electrostatic surfaces of the proteins were calculated and displayed by PyMOL script (http://www.pymol.org). The sequences of S. cerevisiae Pol II largest subunit (RPB1) and E. coli RNAP β’ subunit were aligned by NCBI Blast (http://blast.ncbi.nlm.nih.gov) by composition matrix adjustment method.

Quantitation of sensitivity of Pol II ECs to restriction enzymes

The bands on the gel (Supplementary Fig. 5) were quantified using a PhosphorImager. Cac8I digestion: The amounts of digested DNA present in experimental samples (lanes 5 and 8) were normalized using the control digestion (lane 2). The inverse ratios of the amounts of normalized digested DNA to total amounts of the active ECs were calculated. StyI digestion (lanes 3, 6 and 9): The inverse ratios of the amounts of digested active ECs to total amounts of the active ECs were calculated.
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