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Preparation and Analysis of Positioned Mononucleosomes

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Abstract

Short DNA fragments containing single nucleosomes have been extensively employed as simple model experimental systems for analysis of many intranuclear processes, including binding of proteins to nucleosomes, covalent histone modifications, transcription, DNA repair and ATP-dependent chromatin remodeling. Here we describe several recently developed procedures for obtaining and analysis of mononucleosomes assembled on 200–350-bp DNA fragments.

Keywords

Nucleosomes; chromatin; assembly; methods

1. Introduction

Various processes in eukaryotic nuclei (such as DNA replication, repair, recombination and transcription) occur on DNA organized in chromatin. The minimal structural unit of chromatin is the nucleosome core: 147-bp DNA organized in 1 3/4 superhelical coils on the surface of the histone octamer (1). Short (150–350 bp) DNA fragments containing single nucleosome cores have been extensively employed recently for analysis of many intranuclear processes, including binding of regulatory proteins to nucleosomes (2), histone modifications (3, 4), transcription of nucleosomal templates (5–8), DNA repair in chromatin (9, 10), ATP-dependent chromatin remodeling (11, 12) and analysis of histone chaperones (13) and nucleosome structure and thermodynamics (14–17). In many cases such simple model templates faithfully recapitulate important aspects of these processes (2, 5, 6, 9, 12, 13). At the same time, these experimental systems have the following advantages as compared with more complex polynucleosomal templates: (a) Structure of single positioned nucleosomes and changes in the nucleosome structure during various processes of DNA metabolism can be analyzed with a high resolution. (b) Electrophoretic mobility of mononucleosomes formed on a short DNA fragment (190–350 bp) in a native gel strongly depends on nucleosome position relative to DNA ends (18, 19) and on its histone composition (20, 21). Therefore positioning and histone composition of mononucleosomes that are often changed during various processes can be easily monitored by analysis in a native gel. (c) Since the initial sample often contains only one positioned nucleosome, its fate during various processes can be determined with certainty. (d) The mobility of

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mononucleosomes in a native gel is typically changed upon binding of various protein complexes to the templates. Furthermore, conformationally different complexes having the same protein/DNA stoichiometry often have different mobilities in the gel (21). Thus analysis of protein binding to nucleosomes and protein-induced changes in the conformation of the complexes are relatively straightforward using mononucleosomal templates.

The fate of histones during progression of various processive enzymes (DNA and RNA polymerases) has been extensively studied using various mononucleosomal templates. In some cases nucleosomes remain associated with DNA during progression of the enzymes. Thus on moderately Pol II-transcribed genes, extensive transcription-dependent exchange of H2A/H2B, but not H3/H4, histones was detected (22–27), suggesting that histones H3/H4 never leave DNA during transcription. The underlying Pol II-type mechanism of transcription through chromatin has been recapitulated *in vitro* and is conserved from yeast to human (28). It is characterized by displacement of a single H2A/H2B dimer (29, 30), matching the apparent effect of Pol II passage *in vivo* (24). Remarkably, the subnucleosome (DNA-bound histone hexamer (hexasome) formed upon release of H2A/H2B dimer from the octamer) survives Pol II passage and remains at the original position on DNA (29).

Survival of the subnucleosomes at the original position on DNA suggests an interesting possibility that at least histones H3/H4 never leave the template during Pol II transcription. This possibility is consistent with the lack of exchange of H3/H4 histones *in vivo* (22–27) and with the DNA looping mechanism of Pol II transcription through chromatin observed *in vitro* (31–33). Indeed, histone survival during Pol II transcription *in vitro* is accompanied by formation of a small transient DNA loop ($\emptyset$ -loop) on the surface of histone octamer including molecule of transcribing Pol II (33). During formation of the $\emptyset$ -loop, the recovery of DNA-histone interactions behind Pol II is tightly coupled with their disruption ahead of the enzyme. This coupling is a distinct feature of the Pol II-type mechanism that allows recovery of H3/H4 histones bound at the original position on DNA during transcription (32, 33).

To evaluate whether histones H3/H4 never leave the template during Pol II transcription, the following approach has been utilized (Fig. 1) (34). If the histone octamer was transiently and fully displaced from DNA, the octamer would re-bind to DNA released behind the progressing Pol II and nucleosome positions would be re-equilibrated during transcription (mechanism 1, Fig. 1) (34). If the original nucleosome positions are non-equilibrium, transcription would result in a change in nucleosome positions. Alternatively, if H3/H4 never leave DNA during transcription, nucleosomes would remain at their original positions after transcription, even if these are not the preferential (equilibrated) positions on DNA before transcription (mechanism 2, Fig. 1). Since histones remain associated with DNA during progression of various processive enzymes including ATP-dependent chromatin remodelers and DNA polymerases (see (35) for review), these approaches are likely to be useful beyond the chromatin transcription field.

Below we describe procedures for obtaining and analysis of mononucleosomes in both equilibrated and non-equilibrium positions on 200–350-bp DNA fragments.

2. Materials

2.1. Materials

1. Dialysis membranes (Spectra/Por; molecular weight cut-off of 8000 and 12000–14000).
2. G-25 Quick spin columns (Boehringer Mannheim).
3. Siliconized Eppendorf tubes (PGC Scientific).
4. 3MM chromatography paper (Whatman)
5. QIAquick Gel Extraction kit (Qiagen).
6. Chicken blood (Truslow Farms).
7. CM C-25 Sephadex.
8. Centiprep-30.

2.2. Enzymes

1. T4 polynucleotide kinase (New England Biolabs, NEB).
2. Restriction enzymes (NEB).
3. Klenow fragment of *E. coli* DNA polymerase I (NEB).
4. Taq DNA polymerase (NEB)
5. T4 DNA ligase (NEB)

2.3. Reagents

1. Ethidium bromide (10 mg/ml stock).
2. Butyl alcohol (J.T. Baker).
3. Ethanol (Pharmco)
4. Equilibrated phenol (Sigma).
5. Chloroform (J.T. Baker).
6. β -mercaptoethanol (Sigma).
7. Protease- and nuclease- free BSA (Sigma).
8. Glycerol (J.T. Baker).
9. NP-40 (Calbiochem).
10. Sodium dodecyl sulphate (GibcoBRL).
11. γ -³²P-ATP (3000 or 6000 Ci/mmol; Perkin Elmer)
12. Acrylamide; N,N'-methylene-*bis*-acrylamide (Bis) (Bio-Rad).
13. Glycogen, 10 mg/ml solution (Boehringer Mannheim).

14. Bromophenol Blue dye (Bio-Rad)
15. Xylene Cyanole (Aldrich Chem. Co)
16. Single Stranded Salmon Testes DNA (Sigma)

2.4. Buffers and Solutions

1. PBS buffer: Phosphate-buffered saline (Gibco BRL).
2. Buffer A: 15 mM Tris HCl (pH 7.5), 15 mM NaCl, 60 mM KCl, 0.15 mM spermine, 0.5 mM spermidine, 15 mM β -mercaptoethanol, 0.34 M sucrose, 0.1 mM PMSF.
3. Buffer B: 10 mM Tris HCl (pH 7.5), 350 mM NaCl, 0.5 mM EDTA, 0.1 mM PMSF.
4. NLB (nuclei lysis buffer): 0.25 mM EDTA, 0.1 mM PMSF.
5. TAE buffer: 0.04 M Tris-acetate, pH 8.0, 1 mM EDTA.
6. HE buffer: 10 mM Na-HEPES, pH 8.0, 1 mM EDTA.
7. CRB 1 to 4(Core Reconstitution Buffers): All four buffers contain HE, 5mM mM β -mercaptoethanol, 0.1% NP-40 and NaCl at the following concentrations: Buffer 1 - 1 M; 2 - 0.8 M; 3 - 0.6 M; 4 - 0.01 M.
8. SP6 buffer: 45 mM Na-HEPES, pH 8.0, 6 mM $MgCl_2$, 2 mM spermidine and 2 mg/ml BSA, 10 mM β -mercaptoethanol.
9. 1x TB (transcription buffer): 20 mM Tris HCl (pH 8.0), 5 mM $MgCl_2$, 2 mM β -mercaptoethanol and indicated concentration of KCl, mM.
10. 1x RLB (RNA loading buffer): 95% Formamide, 10 mM EDTA, 0.1% SDS, and 0.01% of each Brome Phenol and Xylene Cyanol dyes.
11. 4x Chromatin Loading Buffer: 100 mM Tris-HCl (pH 7.5), 40 mM EDTA, 40% Sucrose and 1 mg/ml of Salmon Testes DNA.

3. Methods

3.1. Protein purification

We purified hexahistidine-tagged yeast Pol II using published protocols (7) and H2A/H2B and H3/H4 histone pairs were isolated from adult chicken blood by chromatography on hydroxyapatite (36, 37).

3.2. Preparation of donor chromatin from chicken erythrocytes

The protocol described below is a modified version of the method published earlier (6, 39) (*see* Note 1).

¹Donor chromatin isolated from chicken erythrocytes contains mostly unmodified core histones (39). The main advantage of using this source of histones is the minimal presence of proteases in chicken erythrocytes.

3.2.1. Red cell isolation

1. Collect red cells from 200 ml chicken blood by centrifugation at 1800 g for 10 min at 4°C.
2. Carefully remove white cells forming from the top of the pellet.
3. Resuspend cells in 48 ml PBS buffer.
4. Collect red cells by centrifugation at 3000 g for 5 min at 4°C.
5. Repeat steps 3 and 4 two more times.

3.2.2. Nuclei isolation

1. Conduct all manipulations at 4°C with pre-cooled buffers, unless indicated otherwise.
2. Resuspend cells in PBS buffer (half of the volume of the pellet).
3. Add 10 volumes of buffer A supplemented with 0.5% NP-40 and mix by inversion.
4. Collect nuclei by centrifugation at 12000 g for 10 min at 4°C.
5. Re-suspend nuclei in 200 ml of buffer A (no NP-40).
6. Collect nuclei by centrifugation at 12000 g for 10 min at 4°C.
7. Repeat steps 3 and 4 several times until red color disappears.
8. Resuspend purified nuclei in a small volume (50–100 ml) of buffer A.
9. Resuspend ~1 µl of nuclei in 0.9 ml of HE buffer, add 0.1 ml of 10% SDS and measure A_{260} . Concentration of nuclei should be ~200–400 A_{260} /ml.

3.2.3. Chromatin preparation

1. Adjust nuclei concentration to 100 A_{260} /ml.
2. For analytical digestion with micrococcal nuclease (MNase) warm 1 ml of nuclei to 37°C.
3. Add $MgCl_2$ and $CaCl_2$ to 1 mM final concentration.
4. Add 1 µl of MNase to 10 U/ml final concentration and incubate at 37°C.
5. Remove 0.1-ml aliquots after 1, 2, 4, 8, 15, 30 and 60 min and stop the digestion by adding EDTA to 10 mM and SDS to 1% final concentrations.
6. Extract the samples once with one volume of 1:1 (v/v) phenol: chloroform.
7. Precipitate DNA with 3 volumes of ethanol, wash with 70% (v/v) ethanol, dry and dissolve in HE buffer.
8. Analyze DNA in 1 % agarose gel and identify the digestion point where the sizes of DNA fragments are 3–20 kb (Fig. 2, 15 min digestion point was selected).

9. For preparative digestion with MNase warm the nuclei in a 500 ml conical glass flask to 37°C.
10. Add MgCl_2 and CaCl_2 to 1 mM final concentration, MNase to 10 U/ml final concentration, and incubate at 37°C for the required time.
11. Stop the digestion by adding EDTA to 10 mM final concentration.
12. Collect nuclei by centrifugation at 12000 g for 10 min at 4°C.
13. Estimate the volume of the pellet and re-suspend the nuclei in equal volume of NLB buffer (nuclei should become semi-transparent)
14. Collect nuclear debris by centrifugation at 12000 g for 10 min at 4°C.
15. Remove the supernatant containing soluble chromatin (it should be slightly opaque & gray). Discard the pellet.
16. Resuspend an aliquot of chromatin in 0.9 ml of HE buffer, add 0.1 ml of 10% SDS and measure A_{260} . Concentration of chromatin should be ~50–80 A_{260}/ml .

3.2.4. Removal of linker histones H1/H5 from chromatin

1. Adjust concentration of chromatin to 50–100 A_{260}/ml .
2. Pre-soak CM C-25 Sephadex (Pharmacia, 36 mg of resin per mg of chromatin) in buffer B for 1 hr.
3. Slowly add 2 M NaCl to chromatin to 0.35 M final concentration (*see* Note 2).
4. Add 1/3 of pre-soaked CM C-25 Sephadex to chromatin and slowly stir for 2 hrs at 4°C.
5. Collect the resin by centrifugation at 12000 g for 10 min at 4°C. Remove supernatant containing soluble chromatin
6. Repeat steps 4 and 5 two more times.
7. Analyze protein composition of –H1/5 chromatin by SDS-PAGE (Fig. 3). Dissolve ~ 10 ug of histones in 10–60 ul of Laemmli loading buffer. Electrophorese histones in a 18% (acrylamide:bis = (30:0.15)) Laemmli gel (17×17×0.15 cm) for 5–6 hours at 32 mA. Stain the gel with Coomassie Blue.
8. Concentrate –H1 chromatin to ~100 A_{260}/ml (5 mg/ml) on Centriprep-30 by centrifugation at 1300 g for required time at 4°C.
9. Dialyze –H1/5 chromatin overnight against buffer B and store at –70°C (*see* Note 3).

²Chromatin becomes insoluble at 150 mM NaCl and then becomes soluble again when the concentration of NaCl reaches 350 mM.

³Donor –H1 chromatin could be further purified by chromatography on Sephacryl S-400HR (Pharmacia) (40).

3.3. Preparation of DNA fragment for reconstitution

The protocol described below is a modified version of the method published earlier (7).

1. The 204 bp DNA fragment was obtained by PCR amplification using pVT1 5S-containing plasmid as was described previously (38).
2. 5'-end-labeling of the PCR primers with γ [^{32}P] ATP (6000 Ci/mmol) using T4 polynucleotide kinase according to the manufacturer's protocol.
3. PCR-amplify DNA fragments in 500 μl volume (5×100 ml reactions) using Taq DNA polymerase (7).
4. Resolve the obtained DNA fragments in a 1.5 % (w/v) agarose gel containing 0.5 $\mu\text{g/ml}$ ethidium bromide and TAE buffer at 4–6 V/cm for 1.5–3 hours, depending on the resolution required for clear band separation.
5. Using a long wave length UV lamp (to reduce nicking of DNA), identify and excise the required band(s).
6. Purify the fragment using the QIAquick Gel Extraction kit.
7. Extract the samples once with one volume of 1:1 (v/v) phenol: chloroform.
8. Precipitate DNA with 3 volumes of ethanol, wash with 70% (v/v) ethanol, dry and dissolve in 100 μl of HE buffer.
9. Determine DNA concentration by measuring the A_{260} (using $A_{260} = 20$ for 1 mg/ml DNA) and store at -20°C .
10. 204-bp PCR fragment was digested with *TspR1* restriction enzyme for 3h at 65°C .
11. Resolve the obtained DNA fragments in a 1.5 % (w/v) agarose gel containing 0.5 $\mu\text{g/ml}$ ethidium bromide, 4 M urea and TAE buffer at 4–6 V/cm for 1.5–3 hours.
12. Using a long wave length UV lamp (to reduce nicking of DNA), identify and excise the required band(s).
13. Purify the fragment using QIAquick Gel Extraction kit (Qiagen).
14. Extract the sample once with one volume of 1:1 (v/v) phenol: chloroform.
15. Precipitate DNA with 3 volumes of ethanol, wash with 70% (v/v) ethanol, dry and dissolve in 100 μl of HE buffer.
16. Use the 204-bp fragment for reconstitution or ligation with 50-bp promoter fragment for 3 h at 15°C using T4 DNA ligase to obtain the 254-bp DNA fragment (Fig. 4) according to the manufacturer's protocol.
17. The ligated products were gel-purified as a template for reconstitution with donor –H1 chromatin.

3.4. Reconstitution of mononucleosome cores using donor –H1 chromatin before or after ligation

Reconstitution was performed using donor –H1 chromatin or purified histones (see (6) for detail). Nucleosome assembly was performed on the 204-bp DNA fragment and then ligated to the 50-bp promoter-containing fragment, or on the 254-bp DNA fragment that already contains the promoter (Fig. 4 (34)).

1. Cool 500 ml each of CRB1 to CRB4 buffers to 4°C.
2. Mix one to three micrograms of the DNA fragment with long -H1/H5 donor chromatin at a ratio of 1:60 (w:w) in 0.04–0.1 ml of CRB1 buffer (see Note 4).
3. Dialyze successively against CRB2 and CRB3, each for 2 hours at 4°C. Then dialyze the sample against CRB4 for 3 hours or overnight.
4. Transfer the reconstitute to a siliconized Eppendorf tube and store at 4°C (do not freeze).
5. Ligate nucleosomes assembled on 204-bp fragment with 50-bp promoter fragment (17) for 3 h at 15 °C using T4 DNA ligase according to the manufacturer's protocol.
6. Check the samples by analysis by native PAGE (see below).

3.5. Mapping the positions of nucleosome cores by native PAGE

1. Preparation of the sample to load to native gel: the concentration of the reconstituted nucleosomes is determined by the specific activity of the DNA.
2. Loading buffer is added providing a final concentration of 10% sucrose and 50 lg/ml sheared herring testes DNA (Intergen, Purchase, NY).
3. The templates are resolved by native gel electrophoresis (4.5% acrylamide (39:1), 5% glycerol, 20mM Na-HEPES, pH 8, and 0.1mM EDTA) at 100V for 4 h at 4 °C as described (38).
4. Quantitation is performed using a Cyclone Phosphor System (Packard, Meriden, CT).

Nucleosome mobility in the gel is dictated by nucleosome positioning on the 254-bp DNA fragment. Nucleosomes occupy different positions when assembly was conducted before or after DNA ligation (Fig. 4), suggesting that nucleosome occupied non-equilibrium positions when the assembly was conducted before ligation (34). Since these non-equilibrium nucleosome positions are preserved after Pol II transcription (29), the data suggest that histones never leave DNA during this process.

⁴The ratio of DNA to donor chromatin may need to be adjusted because different chromatin preparations produce slightly different results.

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References

1. Luger K, Mader AW, Richmond RK, Sargent DF, Richmond TJ. Crystal structure of the nucleosome core particle at 2.8 Å resolution. *Nature*. 1997; 389:251–260. [PubMed: 9305837]
2. Cirillo LA, Zaret KS. Preparation of defined mononucleosomes, dinucleosomes, and nucleosome arrays *in vitro* and analysis of transcription factor binding. *Methods Enzymol*. 2004; 375:131–158. [PubMed: 14870664]
3. Kim J, Roeder RG. Nucleosomal H2B ubiquitylation with purified factors. *Methods*. 2011; 54:331–338. [PubMed: 21443952]
4. Yun M, Ruan C, Huh JW, Li B. Reconstitution of modified chromatin templates for *in vitro* functional assays. *Methods Mol Biol*. 2012; 833:237–253. [PubMed: 22183598]
5. Gaykalova DA, Kulaeva OI, Pestov NA, Hsieh FK, Studitsky VM. Experimental analysis of the mechanism of chromatin remodeling by RNA polymerase II. *Methods Enzymol*. 2012; 512:293–314. [PubMed: 22910212]
6. Gaykalova DA, Kulaeva OI, Bondarenko VA, Studitsky VM. Preparation and analysis of uniquely positioned mononucleosomes. *Methods Mol Biol*. 2009; 523:109–123. [PubMed: 19381918]
7. Walter W, Studitsky VM. Construction, analysis, and transcription of model nucleosomal templates. *Methods*. 2004; 33:18–24. [PubMed: 15039083]
8. Walter W, Kashlev M, Studitsky VM. Transcription through the nucleosome by mRNA-producing RNA polymerases. *Methods Enzymol*. 2004; 377:445–460. [PubMed: 14979044]
9. Beard BC, Smerdon MJ. Analysis of DNA repair on nucleosome templates. *Methods Enzymol*. 2004; 377:499–507. [PubMed: 14979047]
10. Teng Y, Yu S, Reed SH, Waters R. Lux ex tenebris: nucleotide resolution DNA repair and nucleosome mapping. *Methods*. 2009; 48:23–34. [PubMed: 19269326]
11. Rowe CE, Narlikar GJ. The ATP-dependent remodeler RSC transfers histone dimers and octamers through the rapid formation of an unstable encounter intermediate. *Biochemistry*. 2010; 49:9882–9890. [PubMed: 20853842]
12. Hota SK, Bartholomew B. Approaches for studying nucleosome movement by ATP-dependent chromatin remodeling complexes. *Methods Mol Biol*. 2012; 809:367–380. [PubMed: 22113289]
13. Mizuguchi G, Wu WH, Alami S, Luk E. Biochemical assay for histone H2A.Z replacement by the yeast SWR1 chromatin remodeling complex. *Methods Enzymol*. 2012; 512:275–291. [PubMed: 22910211]
14. Andrews AJ, Luger K. A coupled equilibrium approach to study nucleosome thermodynamics. *Methods Enzymol*. 2011; 488:265–285. [PubMed: 21195232]
15. Dyer PN, Edayathumangalam RS, White CL, Bao Y, Chakravarthy S, Muthurajan UM, Luger K. Reconstitution of nucleosome core particles from recombinant histones and DNA. *Methods Enzymol*. 2004; 375:23–44. [PubMed: 14870657]
16. Luger K, Rechsteiner TJ, Richmond TJ. Preparation of nucleosome core particle from recombinant histones. *Methods Enzymol*. 1999; 304:3–19. [PubMed: 10372352]
17. Vasudevan D, Chua EY, Davey CA. Crystal structures of nucleosome core particles containing the ‘601’ strong positioning sequence. *J Mol Biol*. 2010; 403:1–10. [PubMed: 20800598]
18. Pennings S, Meersseman G, Bradbury EM. Mobility of positioned nucleosomes on 5 S rDNA. *J Mol Biol*. 1991; 220:101–110. [PubMed: 2067009]
19. Meersseman G, Pennings S, Bradbury EM. Mobile nucleosomes—a general behavior. *EMBO J*. 1992; 11:2951–2959. [PubMed: 1639066]
20. Studitsky, VM.; Clark, DJ.; Felsenfeld, G. *Structural Biology: the state of art*. Adenine Press; 1994. Mechanism of nucleosome displacement by a transcribing polymerase; p. 125-131.

21. Kireeva ML, Walter W, Tchernajenko V, Bondarenko V, Kashlev M, Studitsky VM. Nucleosome remodeling induced by RNA polymerase II. Loss of the H2A/H2B dimer during transcription. *Mol Cell*. 2002; 9:541–552. [PubMed: 11931762]
22. Wirbelauer C, Bell O, Schubeler D. 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*. 2005; 19:1761–1766. [PubMed: 16077006]
23. Schwartz BE, Ahmad K. Transcriptional activation triggers deposition and removal of the histone variant H3.3. *Genes Dev*. 2005; 19:804–814. [PubMed: 15774717]
24. Thiriet C, Hayes JJ. Replication-independent core histone dynamics at transcriptionally active loci *in vivo*. *Genes Dev*. 2005; 19:677–682. [PubMed: 15769942]
25. Dion MF, Kaplan T, Kim M, Buratowski S, Friedman N, Rando OJ. Dynamics of replication-independent histone turnover in budding yeast. *Science*. 2007; 315:1405–1408. [PubMed: 17347438]
26. Rufiange A, Jacques PE, Bhat W, Robert F, Nourani A. Genome-wide replication-independent histone H3 exchange occurs predominantly at promoters and implicates H3 K56 acetylation and Asf1. *Mol Cell*. 2007; 27:393–405. [PubMed: 17679090]
27. Jamai A, Imoberdorf RM, Strubin M. Continuous histone H2B and transcription-dependent histone H3 exchange in yeast cells outside of replication. *Mol Cell*. 2007; 25:345–355. [PubMed: 17289583]
28. Bondarenko VA, Steele LM, Ujvari A, Gaykalova DA, Kulaeva OI, Polikanov YS, Luse DS, Studitsky VM. Nucleosomes can form a polar barrier to transcript elongation by RNA polymerase II. *Mol Cell*. 2006; 24:469–479. [PubMed: 17081995]
29. Kireeva ML, Walter W, Tchernajenko V, Bondarenko V, Kashlev M, Studitsky VM. Nucleosome remodeling induced by RNA polymerase II: loss of the H2A/H2B dimer during transcription. *Mol Cell*. 2002; 9:541–552. [PubMed: 11931762]
30. Belotserkovskaya R, Oh S, Bondarenko VA, Orphanides G, Studitsky VM, Reinberg D. FACT facilitates transcription-dependent nucleosome alteration. *Science*. 2003; 301:1090–1093. [PubMed: 12934006]
31. Chang HW, Kulaeva OI, Shaytan AK, Kibanov M, Kuznedelov K, Severinov KV, Kirpichnikov MP, Clark DJ, Studitsky VM. Analysis of the mechanism of nucleosome survival during transcription. *Nucleic Acids Res*. 2014; 42:1619–1627. [PubMed: 24234452]
32. Kulaeva OI, Hsieh FK, Chang HW, Luse DS, Studitsky VM. Mechanism of transcription through a nucleosome by RNA polymerase II. *Biochim Biophys Acta*. 2013; 1829:76–83. [PubMed: 22982194]
33. Kulaeva OI, Gaykalova DA, Pestov NA, Golovastov VV, Vassilyev DG, Artsimovitch I, Studitsky VM. Mechanism of chromatin remodeling and recovery during passage of RNA polymerase II. *Nat Struct Mol Biol*. 2009; 16:1272–1278. [PubMed: 19935686]
34. Kulaeva OI, Studitsky VM. Mechanism of histone survival during transcription by RNA polymerase II. *Transcr*. 2010; 1:85–88.
35. Studitsky VM, Walter W, Kireeva M, Kashlev M, Felsenfeld G. Chromatin remodeling by RNA polymerases. *Trends Biochem Sci*. 2004; 29:127–135. [PubMed: 15003270]
36. Simon RH, Felsenfeld G. A new procedure for purifying histone pairs H2A + H2B and H3 + H4 from chromatin using hydroxylapatite. *Nucleic Acids Res*. 1979; 6:689–696. [PubMed: 424310]
37. von Holt C, Brandt WF, Greyling HJ, Lindsey GG, Retief JD, Rodrigues JD, Schwager S, Sewell BT. Isolation and characterization of histones. *Methods Enzymol*. 1989; 170:431–523. [PubMed: 2549339]
38. Walter W, Kireeva ML, Tchernajenko V, Kashlev M, Studitsky VM. Assay of the fate of the nucleosome during transcription by RNA polymerase II. *Methods Enzymol*. 2003; 371:564–577. [PubMed: 14712729]
39. Ausio J, Dong F, van Holde KE. Use of selectively trypsinized nucleosome core particles to analyze the role of the histone “tails” in the stabilization of the nucleosome. *J Mol Biol*. 1989; 206:451–463. [PubMed: 2716057]

40. Owen-Hughes T, Utley RT, Steger DJ, West JM, John S, Cote J, Havas KM, Workman JL. Analysis of nucleosome disruption by ATP-driven chromatin remodeling complexes. *Methods Mol Biol.* 1999; 119:319–331. [PubMed: 10804522]

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Figure 1. Two possible mechanisms of nucleosome survival during transcription by Pol II
 After Pol II transcription (or progression of other processive enzymes) through chromatin, histones often remain bound to the DNA. Such nucleosome survival could be accompanied either by complete transient displacement (mechanism 1) or only partial displacement of histone octamer from DNA (mechanism 2). According to the mechanism 1 octamer re-binds to DNA released behind the progressing enzyme; in the other case progression of the enzyme is accompanied by formation of an intranucleosomal loop (a small Ø-loop formed during Pol II transcription is shown). As a result, after the enzyme progresses through chromatin, nucleosome positions are either re-equilibrated (mechanism 1), or the original, non-equilibrium nucleosome positions are preserved (mechanism 2).

MNase, min: 1 2 4 8 15 30 60

Figure 2. Time-course of digestion of chicken erythrocyte nuclei with micrococcal nuclease (MNase)

Nuclei were digested for various time intervals (1, 2, 4, 8, 15, 30 or 60 min), DNA purified and analyzed in 1% agarose gel (ethidium bromide stain). 15-min digestion point was selected for preparative digestion of the nuclei (indicated by arrow). M – 100-bp DNA ladder (New England Biolabs).

Figure 3. Analysis of protein composition of histone H1/5-containing and H1/5-depleted chicken erythrocyte chromatin

Histones were resolved by 18% Laemmli SDS-PAGE. Positions of histones on the gel are indicated. M – 10–250 kDa protein markers (BioRad).

Figure 4. Experimental approach for obtaining equilibrated and non-equilibrium sets of mononucleosomes

Nucleosome assembly on the 204-bp DNA fragment was conducted either after (A) or before (B) ligation to the promoter-containing 50-bp fragment. Positions of nucleosomes are equilibrated during, but not after assembly. Therefore in both cases nucleosome positions are equilibrated during the process of assembly, but in (B) ligation to the promoter-containing DNA fragment to the assembled nucleosomes produces templates containing nucleosomes occupying non-equilibrium positions before transcription by Pol II.