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Preparation and Analysis of Uniquely Positioned Mononucleosomes

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Abstract

Short DNA fragments containing single, uniquely positioned nucleosome cores have been extensively employed as simple model experimental systems for analysis of many intranuclear processes, including binding of proteins to nucleosomes, transcription, DNA repair and ATP-dependent chromatin remodeling. In many cases such simple model templates faithfully recapitulate numerous important aspects of these processes. Here we describe several recently developed procedures for obtaining and analysis of mononucleosomes that are uniquely positioned on 150–600 bp DNA fragments.

Keywords

Positioned nucleosome cores; chromatin; reconstitution

1. Introduction

A variety of 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, uniquely positioned nucleosome cores have been extensively employed recently for analysis of many intranuclear processes, including binding of regulatory proteins to nucleosomes (2, 3), transcription of nucleosomal templates (4–7), DNA repair in chromatin (8), ATP-dependent chromatin remodeling (9–13), and analysis of nucleosome structure (14–16). In many cases such simple model templates faithfully recapitulate important aspects of these processes (3, 5, 6, 8, 12, 13, 17). At the same time, this experimental system has the following advantages as compared with polynucleosomal templates: (a) Structure of single, uniquely 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 (17, 20). 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 usually contains only one positioned nucleosome, its fate during various processes can be determined with certainty. (d) The

mobility of mononucleosomes in a native gel often is considerably changed upon binding of various protein complexes to the templates. Often conformationally different complexes having the same protein/DNA stoichiometry have different mobilities in the gel (17). Thus analysis of protein binding to nucleosomes and protein-induced changes in the conformation of the complexes are possible using mononucleosomal templates.

Several strategies could be used to obtain single, uniquely positioned nucleosome cores on a short DNA fragment. Typically, nucleosomes are assembled on a DNA fragment containing a nucleosome-positioning DNA sequence. Natural positioning sequences are not very strong (21). Therefore for DNA fragments that are 150–350-bp long, the result of nucleosome reconstitution usually is a mixture of single, differently positioned nucleosomes. Uniquely positioned nucleosomes can be obtained by reducing the length of DNA fragment to 150 bp (5, 14). If nucleosome-free DNA region has to be present on the template, the nucleosomes formed on 150-bp DNA fragment can be ligated to the nucleosome-free DNA fragment (5). Using this approach, uniquely positioned nucleosomes can be formed even on a random DNA sequence (22). Alternatively, single, differently positioned nucleosomes can be formed on a longer DNA fragment (150–350 bp), and then the templates containing nucleosomes positioned at the desired region of the fragment can be gel purified (23, 24). The method is based on the observation that positional isomers of nucleosome cores have different electrophoretic mobilities (19, 23, 25). A disadvantage of this approach is a low yield of the desired complexes (23).

Most of the technical problems associated with use of weak nucleosome-positioning DNA sequences can be avoided by employing stronger nucleosome-positioning DNA sequences. Particularly strong nucleosome-positioning sequences have been obtained using SELEX approach, starting with a pool of synthetic random-sequence DNA molecules (26, 27). Many of these sequences have affinities for histone binding that are more than six-fold higher than those of the strongest natural positioning sequences, and more than 100-fold higher than the affinity of an “average” DNA sequence (27). The following properties make the high-affinity sequences very useful in vitro: (a) The main advantage of using these sequences is ability to form single, uniquely positioned nucleosomes on the vast majority of DNA molecules present during nucleosome reconstitution (on DNA fragments of 150–600 bp in size). In most cases obtained nucleosome preparations are homogeneous and therefore can be studied without further purification. (b) Knowledge of the consensus DNA sequences determining high DNA-histone affinity and nucleosome positioning (28, 29) allows intelligent design of the templates with desired nucleosome positioning. Since the consensus sequences are localized within the central 70-bp, H3/H4 tetramer-binding region of nucleosomal DNA, the sequences of nucleosomal DNA binding H2A/H2B histone dimers can be modified if needed. (c) Uniquely positioned H3/H4 tetramers can be formed (22). (d) Finally, polynucleosomes containing an array of precisely positioned nucleosomes can be assembled (30).

Several methods for reconstitution of nucleosome cores on DNA have been described (31). In our laboratory we commonly use two methods for nucleosome assembly on the high-affinity DNA sequences. One involves incubation of purified DNA and core histones at high salt followed by reduction in the salt concentration by dialysis. At intermediate salt

concentration H3/H4 bind to DNA as a tetramer and at a lower salt concentration binding of two H2A/H2B dimers results in formation of nucleosome cores (32). In the second approach, the histone octamers are transferred from “donor” chromatin onto DNA (33); however the resulting nucleosome preparation contains some amount of donor chromatin that could affect some reactions. For example, in our laboratory nucleosomes obtained by the transfer method have to be purified from donor chromatin after formation of transcription elongation complexes and their immobilization on a resin (34). Importantly, in both protocols some excess of competitor DNA or donor chromatin is present. The presence of the competitor allows formation of nucleosomes only on the high-affinity DNA sequences of the DNA fragment.

Two methods are the most appropriate for analysis of nucleosome positioning on the high-affinity DNA sequences: low resolution mapping with restriction enzymes and high-resolution mapping using DNase I or hydroxyl radical footprinting (35). Both methods are based on the resistance of nucleosomal DNA to external probes. In the first method, isolated cores are incubated with a restriction enzyme present in the amount sufficient for complete digestion of free DNA and then analyzed in a nucleoprotein gel (36, 37). This method allows rapid mapping of multiple positioned nucleosomes but the resolution is relatively low (~10 bp). It is most useful for quantitative information on the fraction of cores assembled in each position on a DNA fragment, but not for high-resolution analysis of nucleosome positioning. The second method is more involved and requires highly homogeneous preparation of uniquely positioned nucleosomes, but it allows determination of nucleosome positioning with single-nucleotide resolution.

Below we describe several procedures for obtaining and analysis of mononucleosomes that are uniquely positioned on 150–600 bp DNA fragments containing sequences having high affinity to core histones H3/H4 and directing precise nucleosome positioning (28, 29).

2. Materials

2.1. Miscellaneous Items

1. Dialysis membranes (Spectra/Por; molecular weight cut-off of 8000 and 12,000–14,000).
2. G-25 Quick spin columns (Boehringer Mannheim).
3. Siliconized Eppendorf tubes (PGC Scientific).
4. 3 MM 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. Micrococcal nuclease (MNase, Worthington, 10,000 U/ml).
4. Klenow fragment of *Escherichia coli* DNA polymerase I (NEB).
5. Taq DNA polymerase (NEB)
6. DNase I (Sigma)

2.3. 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, and 0.1 mM PMSF.
3. Buffer B: 10 mM Tris-HCl (pH 7.5), 350 mM NaCl, 0.5 mM EDTA, and 0.1 mM PMSF.
4. NLB (nuclei lysis buffer): 0.25 mM EDTA and 0.1 mM PMSF.
5. TAE buffer: 0.04 M Tris-acetate, pH 8.0, and 1 mM EDTA.
6. HE buffer: 10 mM Na-HEPES, pH 8.0, and 1 mM EDTA.
7. CRB 1–6 (core reconstitution buffers): all six buffers contain HE, 5 mM mM β -mercaptoethanol, 0.1% NP-40, and NaCl at the following concentrations: buffer 1–2 M, 2 – 1.2 M, 3 – 1 M, 4 – 0.8 M, 5 – 0.6 M, and 6 – 0.01 M.
8. SP6 buffer: 45 mM Na-HEPES, pH 8.0, 6 mM MgCl₂, 2 mM spermidine, 2 mg/ml BSA, and 10 mM β -mercaptoethanol.
9. 1×TB (transcription buffer): 20 mM Tris-HCl (pH 8.0), 5 mM MgCl₂, 2 mM β -mercaptoethanol, and indicated concentration of KCl, mM.
10. 5 × DSS (DNase I stop solution): 250 mM EDTA and 1% SDS.
11. 40 × Fe (II) stock solution for hydroxyl radical reaction: 80 mM ammonium iron (II) sulfate hexahydrate, (NH₄)Fe(SO₄)₂• 6H₂O.
12. 100 × EDTA stock solution: 0.4 M Na-EDTA.
13. 50 × H₂O₂ stock solution: 30% hydrogen peroxide.
14. 5 × Ascorbate stock solution: 0.1 M sodium salt of L-ascorbic acid.
15. 1 × RLB (RNA loading buffer): 95% formamide, 10 mM EDTA, 0.1% SDS, and 0.01% of each bromophenol blue and xylene cyanol dyes.
16. 10 × HRSS (hydroxyl radical stop solution): 0.1 M thiourea, 0.3 M Na-acetate, 30 mM EDTA, and 2 mg/ml glycogen.
17. 4 × Chromatin loading buffer: 100 mM Tris-HCl (pH 7.5), 40 mM EDTA, 40% sucrose, and 1 mg/ml of Salmon Testes DNA.

2.4. Proteins

1. H2A/H2B and H3/H4 histone pairs isolated from adult chicken blood by chromatography on hydroxyapatite (32, 38).

3. Methods

3.1. Preparation of Short DNA Templates Containing a Strong Nucleosome-Positioning Sequence

1. Design the short DNA template (*see* Note 1).
2. 5'-end-label one of the PCR primers with γ [^{32}P] ATP (6000 Ci/mmol) using T4 polynucleotide kinase (*see* Note 2).
3. PCR-amplify DNA fragments in 500 μl volume (5×100 ml reactions) using Taq DNA polymerase (New England Biolabs).
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 h, depending on the resolution required for clear band separation.
5. Using a long wavelength UV lamp (to reduce nicking of DNA), identify and excise the required band(s).
6. Purify the fragment using QIAquick Gel Extraction kit (Qiagen).
7. Extract the samples once with one volume of 1:1 (v/v) phenol:chloroform.
8. Precipitate DNA with three 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 .

3.2. Preparation of Donor Chromatin from Chicken Erythrocytes

3.2.1. Red Cell Isolation—The protocol described below is a modified version of the method published earlier (39) (*see* Note 3).

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.

¹A strong nucleosome-positioning sequence can be placed at any location within the DNA fragment. A single, precisely positioned nucleosome can be formed on DNA fragments up to at least 600 bp long.

²For footprinting studies only one DNA strand must be endlabeled. The 5'-end of the fragment can be end labeled by the protein kinase and the 3'-end – by the Klenow fragment (23). To determine rotational orientation of DNA on the surface of the histone octamer, two nucleosome preparations containing different labeled DNA strands need to be prepared.

³Donor chromatin isolated from chicken erythrocytes contains mostly unmodified core histones (39). The main advantage of using this source of histones is the presence of low amount of proteases in chicken erythrocytes.

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 12,000 g for 10 min at 4°C.
5. Resuspend nuclei in 200 ml of buffer A (no NP-40).
6. Collect nuclei by centrifugation at 12,000 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 A₂₆₀. Concentration of nuclei should be ~200–400 A₂₆₀/ml.

3.2.3. Chromatin Preparation

1. Adjust nuclei concentration to 100 A₂₆₀/ml.
2. For analytical digestion with micrococcal nuclease (MNase) warm 1 ml of nuclei to 37°C.
3. Add MgCl₂ and CaCl₂ 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 concentration.
6. Extract the samples once with one volume of 1:1 (v/v) phenol:chloroform.
7. Precipitate DNA with three 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. 8.1, 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 MgCl₂ and CaCl₂ 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 12,000 g for 10 min at 4°C.
13. Estimate the volume of the pellet and resuspend the nuclei in equal volume of NLB buffer (nuclei should become semitransparent).
14. Collect nuclear debris by centrifugation at 12,000 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 h.
3. Slowly add 2 M NaCl to chromatin to 0.35 M final concentration (*see* Note 4).
4. Add 1/3 of pre-soked CM C-25 Sephadex to chromatin and slowly stir for 2 h at 4°C.
5. Collect the resin by centrifugation at 12,000 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. 8.2). Dissolve ~10 µg of histones in 10–60 µl of Laemmli loading buffer. Electrophorese histones in a 18% (acrylamide:bis = (30:0.15)) Laemmli gel (17 × 17 × 0.15 cm) for 5–6 h 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 –7°C (*see* Note 5).

3.3. Reconstitution of Mononucleosome Cores on High-Affinity DNA Sequences

3.3.1. Reconstitution of Mononucleosome Cores Using Ppurified Core Histones

1. Cool 500 ml each of CRB1 to CRB6 buffers to 4°C.
2. Dissolve 0.5–3 µg of DNA in the CRB1 buffer.
3. Add Salmon Testes DNA to achieve two-fold weight excess over the amount of the DNA fragment and purified core histones to a molar ratio of 1.5–2.0 of core histone octamer to DNA (use 1.5 excess of H2A/H2B dimer, *see* Note 6). Adjust volume to 40–100 µl.
4. Dialyze against CRB1 at 4°C overnight.
5. Next morning, dialyze successively against CRB2, CRB3, CRB4, and CRB5, each for 1 h. Then dialyze against CRB6 for 3 h or overnight.

⁴Chromatin becomes insoluble at 150 mM NaCl and then becomes soluble again when the concentration of NaCl reaches 350 mM.

⁵Donor –H1 chromatin could be further purified by chromatography on Sephacryl S-400HR (Pharmacia) (33).

⁶The presence of the excess of H2A/H2B histones guarantees formation of complete nucleosomes. We found that at stoichiometric ratio of H2A/H2B to H3/H4 histones some amount of incomplete nucleosomes (primarily missing one H2A/H2B dimer) is formed.

6. Transfer the reconstitute to a siliconized Eppendorf tube (*see* Note 7) and store at 4°C (do not freeze).
7. Check the quality of reconstitution by native 4.5% PAGE (23). Electrophorese the cores in a 4.5% (acrylamide:bis = 40:1) gel containing 20 mM HEPES (pH 8.0), 1 mM EDTA, and 5% (v/v) glycerol (17 × 17 × 0.15 cm) for 2–3 h at 6 V/cm.
8. Dry the gel on Whatman 3MM paper and expose with a PhosphorImager screen at room temperature for 2–3 h or overnight if necessary. Assembled nucleosomes are expected to migrate as a single band in the gel (Fig. 8.3).

3.3.2. Reconstitution of the Mononucleosome Cores Using Donor –H1 Chromatin

1. Cool 500 ml each of CRB4 to CRB6 buffers to 4°C.
2. Mix 1–3 µg of the DNA fragment with long –H1/H5 donor chromatin at a ratio of 1:5 (w:w) in 0.04–0.1 ml of CRB3 buffer (*see* Note 8).
3. Dialyze successively against CRB4 and CRB5, each for 2 h at 4°C. Then dialyze the sample against CRB6 for 3 h or overnight.
4. Transfer the reconstitute to a siliconized Eppendorf tube (*see* Note 7) and store at 4°C (do not freeze).
5. Check the quality of reconstitution by analysis by native 4.5% PAGE (23). Assembled nucleosomes are expected to migrate as a single band.

3.4. Mapping the Positions of Nucleosome Cores

3.4.1. Mapping with Restriction Enzymes

1. Digest 20 ng (DNA) of labeled cores in 20 µl of SP6 buffer with 5–10 U of corresponding restriction enzyme at 37°C for 20 min (*see* Note 9). If needed, add labeled DNA to the sample as an intrinsic control for enzyme activity.
2. Stop the reaction by adding 1/3 volume of 4× chromatin loading buffer. Analyze the digests by native 4.5% PAGE.
3. Dry the gel on Whatman 3MM paper and expose with a PhosphorImager screen at room temperature for 2–3 h or overnight if necessary. An example of restriction enzyme mapping of nucleosome core positions is shown in Fig. 8.4.

3.4.2. Mapping with DNase I endonuclease

1. Use 20–100 ng (DNA) of DNA-end-labeled nucleosome or histone-free DNA (10,000–50,000 cpm) per reaction in 20 µl of TB40 buffer (*see* Note 10).

⁷Use of siliconized tubes is essential to prevent nucleosome disruption during storage at low concentration (1–10 µg/ml). Presumably, siliconization prevents irreversible adsorption of histones to the walls of the tube.

⁸The ratio of DNA to donor chromatin may need to be adjusted because different chromatin preparations produce slightly different results.

⁹Recognition site for the restriction enzyme must be present within the region covered by nucleosome in the desired position. We found that restriction enzyme sites are protected even when they are located 5 bp in the nucleosome core (31).

2. Add 0.1–0.2 units of DNase I per 100 ng of total DNA in the reaction (including Shredded Salmon Sperm DNA that could be present in the reconstitute). Incubate for 30 s at 37°C (*see* Note 11).
3. Stop reaction by adding ¼ volumes of 5× DSS.
4. Adjust volume to 0.1 ml and extract the samples once with one volume of 1:1 (v/v) phenol:chloroform.
5. Precipitate DNA with three volumes of ethanol, wash with 70% (v/v) ethanol, dry, and dissolve in 5 µl of RLB buffer. Denature samples at 95°C.
6. Electrophorese DNA in an 8% (acrylamide:bis 19:1) gel containing 8 M urea and 0.5× TBE buffer (17 × 34 0.03 cm) for 40 min at 2000 V.
7. Dry gel on Whatman 3MM paper and expose to a Phosphor-Imager screen at room temperature overnight. An example of DNase I footprinting of a positioned nucleosome is shown in Fig. 8.5A.

3.4.3. Mapping with Hydroxyl Radicals

1. The efficiency of DNA cleavage by hydroxyl radicals does not depend on DNA concentration, but is strongly inhibited by some chemicals: MgCl₂ (> 20 mM), glycerol (>0.1%), and β-mercaptoethanol (>1 mM) (35). Therefore before hydroxyl radical footprinting the samples have to be dialyzed against a buffer that does not contain these chemicals.
2. Use 20–100 ng (DNA) of DNA-end-labeled nucleosomes or histone-free DNA (10,000–50,000 cpm) per reaction in 50 µl of HE buffer (the amount of nucleosomes should be twice the amount of histone-free DNA).
3. Prepare the following solutions just before the experiment: (a) 2 mM ammonium iron (II) sulfate hexahydrate – 4 mM EDTA, (b) 0.6% hydrogen peroxide, and (c) 20 mM sodium ascorbate.
4. Put siliconized Eppendorf tubes containing nucleosomes or histone-free DNA on the side and place 3.3-, 6.6-, or 9.9 µl drops each of the solutions on the wall of the tube. Quickly mix all components and incubate 2 min at room temperature (*see* Note 12).
5. Stop reactions by adding 6.6, 7.7 or 8.8 µl of 10× HR stop solution.
6. Adjust volume to 100 µl and extract the samples once with one volume of 1:1 (v/v) phenol:chloroform.
7. Precipitate DNA with three volumes of ethanol, wash with 70% (v/v) ethanol, dry, and dissolve in 5 µl of RLB buffer. Denature samples for 1–2 min at 95°C.

¹⁰For both DNase I and hydroxyl radical footprinting techniques it is essential to compare the results of footprinting of nucleosomes and histone-free DNA (Fig. 8.5). Therefore end-labeled nucleosomes and histone-free DNA samples should be prepared in parallel.

¹¹Addition of different amounts of DNase I results in variable extents of DNA cleavage. The fraction of DNA molecules containing the cleavages should not exceed 30% to guarantee that only one cleavage per molecule is introduced.

¹²Addition of different amounts of the reagents results in variable extents of DNA cleavage. The fraction of DNA molecules containing the cleavages should not exceed 30% to guarantee that only one cleavage per molecule is introduced.

8. Electrophorese DNA in an 8% (acrylamide:bis = 19:1) gel containing 8 M urea and 0.5× (17 × 34 × 0.03 cm) for 40 min at 2000 V. Use sequencing markers if high accuracy is needed
9. Dry gel on Whatman 3MM paper and expose to a Phosphor-Imager screen at room temperature overnight. An example of hydroxyl radical footprinting of a positioned nucleosome is shown in Fig. 8.5B.

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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. Utley RT, Owen-Hughes TA, Juan LJ, Cote J, Adams CC, Workman JL. *In vitro* analysis of transcription factor binding to nucleosomes and nucleosome disruption/displacement. *Meth Enzymol*. 1996; 274:276–291. [PubMed: 8902812]
3. Cirillo LA, Zaret KS. Preparation of defined mononucleosomes, dinucleosomes, and nucleosome arrays *in vitro* and analysis of transcription factor binding. *Meth Enzymol*. 2004; 375:131–158. [PubMed: 14870664]
4. Studitsky VM, Clark DJ, Felsenfeld G. Preparation of nucleosomal templates for transcription *in vitro*. *Meth Enzymol*. 1996; 274:246–256. [PubMed: 8902809]
5. Walter W, Studitsky VM. Construction, analysis, and transcription of model nucleosomal templates. *Methods*. 2004; 33:18–24. [PubMed: 15039083]
6. Walter W, Kashlev M, Studitsky VM. Transcription through the nucleosome by mRNA-producing RNA polymerases. *Meth Enzymol*. 2004; 377:445–460. [PubMed: 14979044]
7. Walter W, Kireeva ML, Tchernajenko V, Kashlev M, Studitsky VM. Assay of the fate of the nucleosome during transcription by RNA polymerase II. *Meth Enzymol*. 2003; 371:564–577. [PubMed: 14712729]
8. Beard BC, Smerdon MJ. Analysis of DNA repair on nucleosome templates. *Meth Enzymol*. 2004; 377:499–507. [PubMed: 14979047]
9. Wittmeyer J, Saha A, Cairns B. DNA translocation and nucleosome remodeling assays by the RSC chromatin remodeling complex. *Meth Enzymol*. 2004; 377:322–343. [PubMed: 14979035]
10. Lorch Y, Kornberg RD. Isolation and assay of the RSC chromatin-remodeling complex from *Saccharomyces cerevisiae*. *Meth Enzymol*. 2004; 377:316–322. [PubMed: 14979034]
11. Eberharder A, Langst G, Becker PB. A nucleosome sliding assay for chromatin remodeling factors. *Meth Enzymol*. 2004; 377:344–353. [PubMed: 14979036]
12. Kassabov SR, Bartholomew B. Site-directed histone-DNA contact mapping for analysis of nucleosome dynamics. *Meth Enzymol*. 2004; 375:193–210. [PubMed: 14870668]
13. Mizuguchi G, Shen X, Landry J, Wu WH, Sen S, Wu C. ATP-driven exchange of histone H2AZ variant catalyzed by SWR1 chromatin remodeling complex. *Science*. 2004; 303:343–348. [PubMed: 14645854]
14. Dyer PN, Edayathumangalam RS, White CL, Bao Y, Chakravarthy S, Muthurajan UM, Luger K. Reconstitution of nucleosome core particles from recombinant histones and DNA. *Meth Enzymol*. 2004; 375:23–44. [PubMed: 14870657]
15. Luger K, Rechsteiner TJ, Richmond TJ. Preparation of nucleosome core particle from recombinant histones. *Meth Enzymol*. 1999; 304:3–19. [PubMed: 10372352]
16. Hanson BL, Alexander C, Harp JM, Bunick GJ. Preparation and crystallization of nucleosome core particle. *Meth Enzymol*. 2004; 375:44–62. [PubMed: 14870658]

17. 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]
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. Widom J. Role of DNA sequence in nucleosome stability and dynamics. *Q Rev Biophys*. 2001; 34:269–324. [PubMed: 11838235]
22. Bondarenko VA, Steele LM, Ujvari A, Gaykalova D, Kulaeva OI, Polykanov YS, Luse DS, Studitsky VM. Nucleosomes Can Form a Polar Barrier to Transcript Elongation by RNA Polymerase II. *Molecular Cell*. 2006; 24:469–479. [PubMed: 17081995]
23. Studitsky VM. Preparation and analysis of positioned nucleosomes. *Meth Mol Biol*. 1999; 119:17–26.
24. Studitsky VM, Clark DJ, Felsenfeld G. Overcoming a nucleosomal barrier to transcription. *Cell*. 1995; 83:19–27. [PubMed: 7553869]
25. Duband-Goulet I, Carot V, Ulyanov AV, Douc-Rasy S, Prunell A. Chromatin reconstitution on small DNA rings. IV DNA supercoiling and nucleosome sequence preference. *J Mol Biol*. 1992; 224:981–1001. [PubMed: 1314907]
26. Lowary PT, Widom J. New DNA sequence rules for high affinity binding to histone octamer and sequence-directed nucleosome positioning. *J Mol Biol*. 1998; 276:19–42. [PubMed: 9514715]
27. Thastrom A, Lowary PT, Widlund HR, Cao H, Kubista M, Widom J. Sequence motifs and free energies of selected natural and non-natural nucleosome positioning DNA sequences. *J Mol Biol*. 1999; 288:213–229. [PubMed: 10329138]
28. Wang JP, Widom J. Improved alignment of nucleosome DNA sequences using a mixture model. *Nucleic Acids Res*. 2005; 33:6743–6755. [PubMed: 16339114]
29. Thastrom A, Bingham LM, Widom J. Nucleosomal locations of dominant DNA sequence motifs for histone–DNA interactions and nucleosome positioning. *J Mol Biol*. 2004; 338:695–709. [PubMed: 15099738]
30. Dorigo B, Schalch T, Kulangara A, lDuda S, Schroeder RR, Richmond TJ. Nucleosome arrays reveal the two-start organization of the chromatin fiber. *Science*. 2004; 306:1571–1573. [PubMed: 15567867]
31. Rhodes D, Laskey RA. Assembly of nucleosomes and chromatin *in vitro*. *Meth Enzymol*. 1989; 170:575–585. [PubMed: 2770552]
32. 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]
33. 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. *Meth Mol Biol*. 1999; 119:319–331.
34. 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]
35. Schickor P, Heumann H. Hydroxyl radical footprinting. *Meth Mol Biol*. 1994; 30:21–32.
36. Studitsky VM, Clark DJ, Felsenfeld G. A histone octamer can step around a transcribing polymerase without leaving the template. *Cell*. 1994; 76:371–382. [PubMed: 8293470]
37. Morse RH. Nucleosomes inhibit both transcriptional initiation and elongation by RNA polymerase III *in vitro*. *EMBO J*. 1989; 8:2343–2351. [PubMed: 2792088]
38. von Holt C, Brandt WF, Greyling HJ, Lindsey GG, Retief JD, Rodrigues JD, Schwager S, Sewell BT. Isolation and characterization of histones. *Meth Enzymol*. 1989; 170:431–523. [PubMed: 2549339]

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]

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Fig. 8.1.

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). A 15 min digestion point was selected for preparative digestion of the nuclei (indicated by arrow). M – 100-bp DNA ladder (New England Biolabs).

Fig. 8.2.

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 kD protein markers (BioRad).

Fig. 8.3.

Analysis of end-labeled 157-bp DNA and nucleosomes by native 4.5% PAGE. Nucleosomes are analyzed after reconstitution on the 601 high-affinity DNA sequence (27) without further purification. Note that nucleosome preparations are homogeneous and do not contain considerable amount of contaminating histone-free DNA. Positions of DNA and nucleosomes in the gel are indicated. M – end-labeled pBR322-*Msp* I digest (New England Biolabs).

Fig. 8.4.

Analysis of nucleosome positioning on 220 bp DNA fragment using the restriction enzyme sensitivity assay. The DNA fragment contains the 601 high-affinity DNA sequence (27). If nucleosomes are positioned symmetrically, they have the same mobilities in a native gel, but are differentially sensitive to restriction enzymes and therefore can be resolved in a native gel after incubation with the enzymes. *Top*: restriction map of the 220 bp 601 template. The template was end-labeled (indicated by the *asterisk*). The cleavage sites for *AflIII*, *StyI*, *AluI*, and *EagI* and the expected position of nucleosome core (*oval*) are indicated. *Bottom*: analysis of nucleosome cores in the native 4.5% PAGE. Mobilities of nucleosomes and histone-free DNA are indicated. Cores were digested with *AflIII*, *StyI*, *AluI*, or *EagI* as indicated. M, end-labeled *MspI* digest of pBR322. Note that some amount of histone-free DNA was added to nucleosomal templates to serve as an internal control for the efficiency of digestion. All enzymes completely digested histone-free DNA.

Fig. 8.5.

Fine mapping of translational positions of 605 nucleosome cores (27) and rotational orientation of DNA on the surface of the octamer using footprinting. **A.** DNase I footprinting. A 256 bp histone-free end-labeled DNA fragment or nucleosome cores were incubated with DNase I, DNA purified, and analyzed by denaturing PAGE. The predominant position of nucleosome core is indicated by oval. M, end-labeled *MspI* digest of pBR322. **B.** Hydroxyl radical footprinting. A 217 bp histone-free end-labeled DNA fragment or nucleosome cores were incubated in the presence of hydroxyl radicals, DNA purified, and analyzed by denaturing PAGE. Note that the expected 10 bp periodicity of DNA cutting is not immediately obvious after treatment with DNase I because of the sequence specificity of the enzyme. At the same time, hydroxyl radicals produce clear 10 bp periodic pattern allowing determination of precise rotational orientation of DNA on the surface of the octamer.