Title

Prenucleosomes and Active Chromatin

Authors

Khuong, Mai T

Fei, Jia

Ishii, Haruhiko

et al.

Publication Date

2015

DOI

10.1101/sqb.2015.80.027300

Peer reviewed

Prenucleosomes and Active Chromatin

Mai T. Khuong,[1,3] Jia Fei,[1,3] Haruhiko Ishii,[2] and James T. Kadonaga[1]
[1]*Section of Molecular Biology, University of California, San Diego, La Jolla, California 92093-0347*
[2]*Ludwig Institute for Cancer Research, San Diego Branch, La Jolla, California 92093-0653*
Correspondence: jkadonaga@ucsd.edu

Chromatin consists of nucleosomes as well as nonnucleosomal histone-containing particles. Here we describe the prenucleosome, which is a stable conformational isomer of the nucleosome that associates with ~80 bp DNA. Prenucleosomes are formed rapidly upon the deposition of histones onto DNA and can be converted into canonical nucleosomes by an ATP-driven chromatin assembly factor such as ACF. Different lines of evidence reveal that there are prenucleosome-sized DNA-containing particles with histones in the upstream region of active promoters. Moreover, p300 acetylates histone H3K56 in prenucleosomes but not in nucleosomes, and H3K56 acetylation is found at active promoters and enhancers. These findings therefore suggest that there may be prenucleosomes or prenucleosome-like particles in the upstream region of active promoters. More generally, we postulate that prenucleosomes or prenucleosome-like particles are present at dynamic chromatin, whereas canonical nucleosomes are at static chromatin.

The nucleosome is the basic repeating unit of chromatin in the nuclei of eukaryotic cells. The nucleosome core consists of ~147 bp DNA that is wrapped approximately 1.7 turns around a histone octamer, which contains two copies each of histones H2A, H2B, H3, and H4. Native chromatin in the cell additionally contains ~50 bp of linker DNA between the nucleosome cores as well as other components such as histone H1 and high-mobility group (HMG) proteins.

In addition to the nucleosome, it has been increasingly appreciated that there are nonnucleosomal histone-containing particles in chromatin (Fig. 1A). These noncanonical chromatin particles have been observed at locations, such as active promoter regions, where nucleosomes are disrupted. Biochemical data have also revealed the existence of the prenucleosome, a stable nonnucleosomal histone-containing particle that can be converted into a canonical nucleosome by an ATP-driven motor protein such as ACF (APOBEC1 complementation factor). We postulate that the noncanonical particles reflect dynamic activity in chromatin, whereas canonical nucleosomes occur in relatively static chromatin. Here, we discuss the identification and characterization of the prenucleosome, a conformational isomer of the nucleosome (Torigoe et al. 2011; Fei et al. 2015).

DISCOVERY OF THE PRENUCLEOSOME AS A PRECURSOR TO THE NUCLEOSOME

We initially found the prenucleosome in the course of our studies on chromatin assembly (Torigoe et al. 2011). A simplified depiction of the steps of the chromatin assembly process (Torigoe et al. 2013) is shown in Figure 1B. First, the core histones are synthesized in the cytoplasm and imported into the nucleus in a complex with one of a variety of histone chaperone proteins. In a fast (complete in <15 sec) reaction, the histones are rapidly and spontaneously deposited onto free DNA to give randomly distributed prenucleosomes. Next, in a slower step (complete in ~10–15 min), an ATP-dependent motor protein, such as ACF or Chd1, converts the prenucleosomes to canonical nucleosomes. Then, the randomly distributed nucleosomes are converted into periodic nucleosome arrays by the ATP-dependent motor protein, which can function both as a chromatin assembly factor (for the conversion of prenucleosomes to nucleosomes) and as a chromatin remodeling factor (for the mobilization of nucleosomes into periodic arrays). The organization of the nucleosomes into periodic arrays is likely to be driven by interactions between nucleosomes (such as chromatin folding and compaction) that result in a thermodynamically more stable arrangement with evenly spaced nucleosomes than with randomly distributed nucleosomes.

Prenucleosomes were discovered in the analysis of the deposition of histones onto DNA during chromatin assembly (Torigoe et al. 2011). In these experiments, we used a DNA template association assay (Fig. 1C) as follows: (1) Histone–chaperone complexes are added to Template 1; (2) Template 2 is added; and (3) ATP-dependent nucleosome assembly is mediated by ACF. If stable histone–Template 1 complexes are formed, then nucleosomes would be assembled on Template 1 but not on Template 2. If the histone–Template 1 complex is not stable, then nucleosomes would be formed on both Template 1 and Template 2. With the NAP1 histone chaperone, we observed template association as shown in Figure 1C (Torigoe et al. 2011). Prenucleosomes were identified on the basis of their ability to remain associated with Template 1 before nucleosome assembly by ACF.

We additionally sought to test whether template association can be seen with other core histone chaperones.

[3]These authors contributed equally to this work.

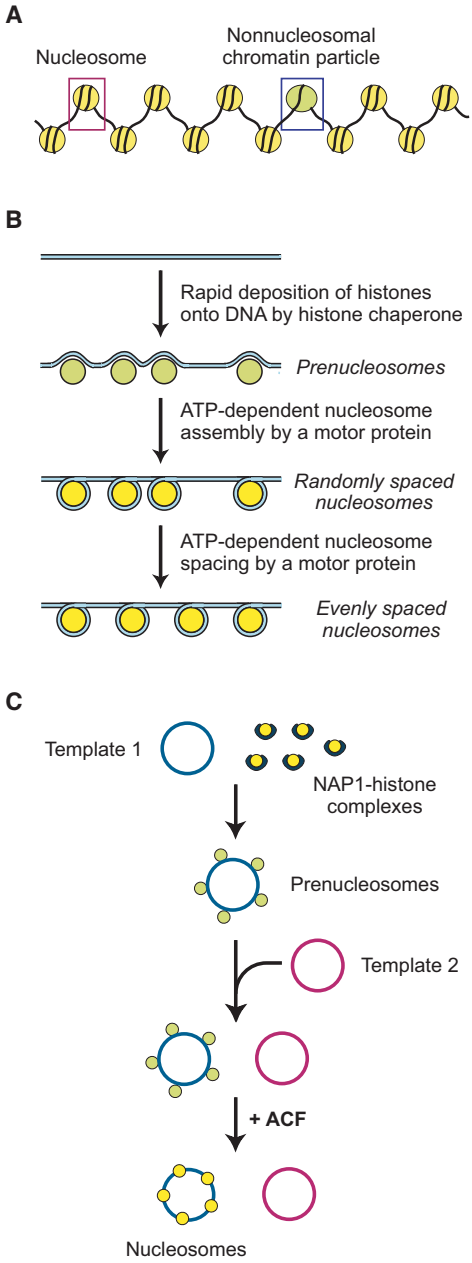

Figure 1. The prenucleosome was initially discovered as a precursor to the nucleosome. (*A*) Chromatin contains nucleosomes as well as nonnucleosomal histone–DNA particles. (*B*) A simple working model for the steps in the assembly of chromatin. This model is based on results from Torigoe et al. (2011, 2013). (*C*) Diagram of the template association assay for chromatin assembly. This assay is described in the text.

To this end, we tested the properties of *Drosophila* nucleoplasmin-like protein, dNLP (Ito et al. 1996). First, we purified dNLP protein (Fig. 2A) and found that it is able to function along with purified ACF in the ATP-dependent assembly of chromatin (Fig. 2B). We then performed template association experiments, as depicted in Figure 1C, and observed the formation of template-associated prenucleosomes with dNLP (Fig. 2C). These experiments therefore show that prenucleosomes can be formed with the dNLP histone chaperone. Hence, the generation of prenucleosomes is not a special property of NAP1 (specifically, *Drosophila* NAP1, as used in Torigoe et al. 2011).

The formation of prenucleosomes and their conversion to nucleosomes can be seen by focusing on the two blue rectangles in Figure 2C (lanes 2 and 4). (As a technical note, in Fig. 2B,C, we monitored chromatin assembly by using the DNA supercoiling assay, in which the formation of nucleosomes is observed by the generation of negative supercoils in the associated circular plasmid DNA [see, e.g., Fyodorov and Kadonaga 2003].) In the absence of ACF (lanes 1 and 2), there is a small increase in DNA supercoiling during the 30-min chromatin assembly reaction, whereas in the presence of ACF (lanes 3 and 4), there is a large increase in supercoiling that is due to the formation of nucleosomes. The key comparison, however, is between the two blue rectangles. In the absence of ACF (lane 2), the histones are associated with Template 1 (the 3K template), but the histone–DNA species (i.e., mostly prenucleosomes) do not substantially supercoil the DNA. In the presence of ACF (lane 4), the histones, which are specifically associated with Template 1, are assembled into nucleosomes by ACF, as seen by the DNA supercoiling. Thus, in the absence of ACF, prenucleosomes (which do not supercoil DNA) are specifically associated with Template 1; then, upon addition of ACF, the prenucleosomes are converted to nucleosomes.

The initial characterization of prenucleosomes revealed the following (Torigoe et al. 2011).

- Prenucleosomes contain all four core histones and lack the NAP1 chaperone.
- Unlike nucleosomes, prenucleosomes do not induce supercoiling of the DNA.
- Prenucleosomes can be converted into nucleosomes by the ACF motor protein.
- The rate of ACF-mediated conversion of prenucleosomes to nucleosomes is comparable to the overall rate of ACF-catalyzed chromatin assembly.
- Prenucleosomes are rapidly formed in the absence of ACF. The formation of prenucleosomes is complete within 15 sec, which is the shortest time point that could be measured in a standard assay. This effect was previously shown with NAP1–histone complexes (Torigoe et al. 2011). We have also seen the rapid formation of prenucleosomes with dNLP–histone complexes (Fig. 3A).
- When a competing free DNA template is added to a prenucleosomal template, the prenucleosomes remain stably associated with the original template for at least 2 h. This property, which was originally seen with NAP1 (Torigoe et al. 2011), is also observed with dNLP as the histone chaperone (Fig. 3B).
- When NAP1–histone complexes are added to relaxed circular DNA, a mixture of ~85% prenucleosomes and 15% nucleosomes is formed. The apparently sponta-

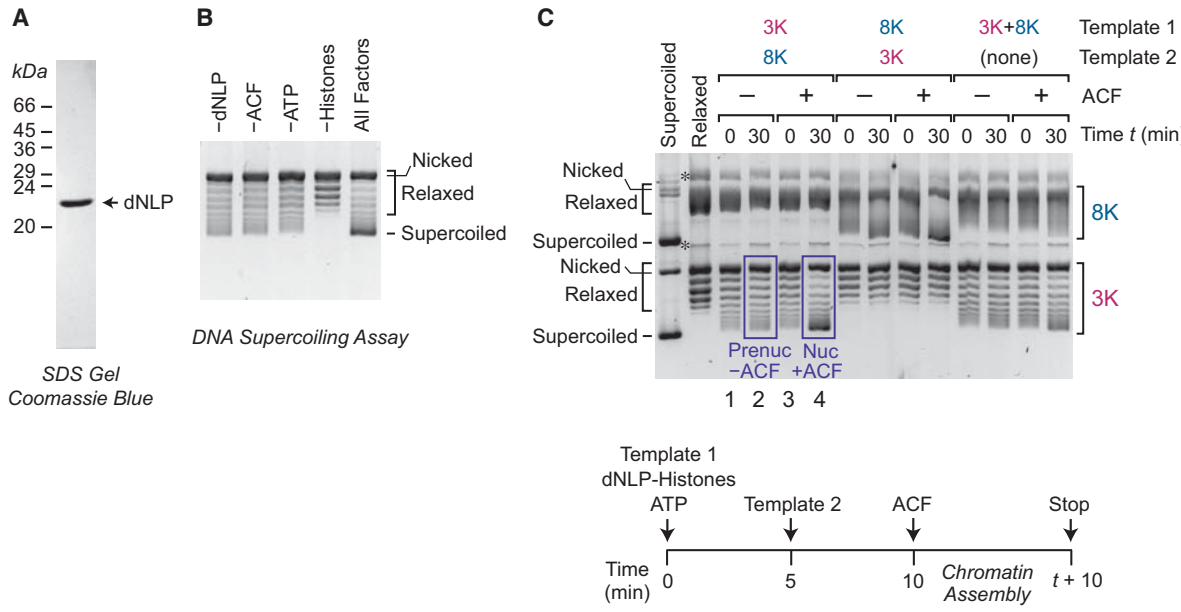

Figure 2. Template association assay with the dNLP core histone chaperone. (*A*) Purification of recombinant dNLP by the method of Ito et al. (1996). (*B*) Chromatin assembly requires dNLP, ACF, core histones, and ATP. Chromatin assembly was performed and monitored by the DNA supercoiling assay, as described in Fyodorov and Kadonaga (2003). (*C*) Template association experiments were performed with equivalent masses of two different relaxed circular DNA templates of ~3 kb (3K) and 8 kb (8K). Core histones (amount sufficient for complete assembly of one DNA template) were initially incubated with Template 1. After 5 min, Template 2 was added to the mixture, which was then allowed to incubate for another 5 min (to allow potential exchange of histones from Template 1 to Template 2) before the addition of ACF. Purified topoisomerase I was included in the reactions, and the formation of nucleosomes was monitored by the DNA supercoiling assay. The 3K and 8K templates were resolved by agarose gel electrophoresis. As references, supercoiled and relaxed DNAs are also included. The positions of relaxed, supercoiled, and nicked DNA species are shown. The asterisks indicate contaminants in the plasmid DNA preparations. The blue rectangles that highlight prenucleosomes (Prenuc) and nucleosome (Nuc) are discussed in the text.

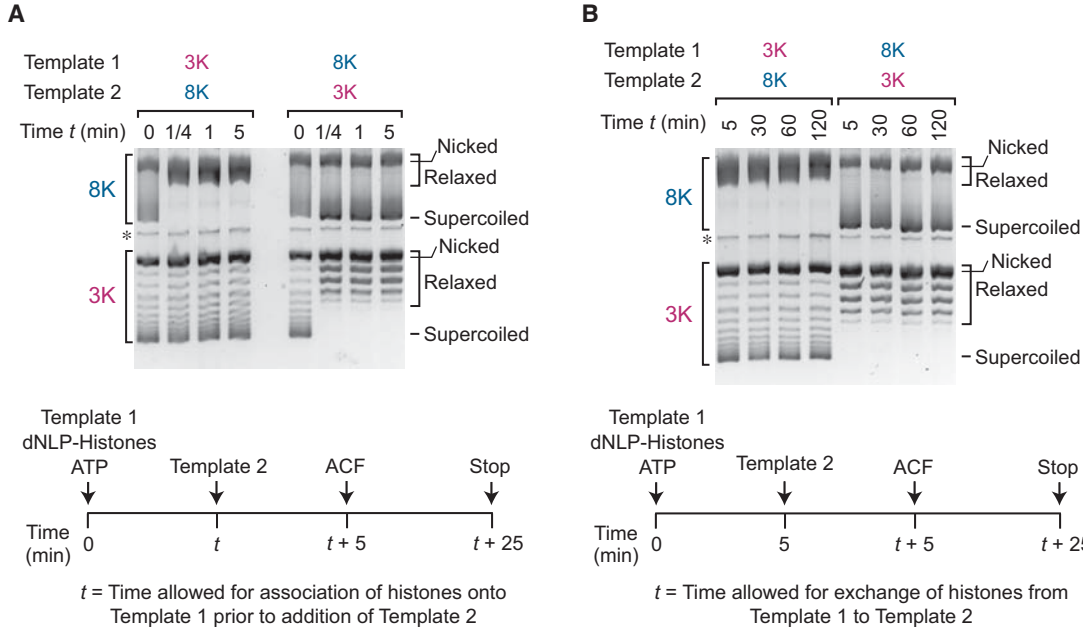

Figure 3. Analysis of the formation and stability of prenucleosomes. (*A*) Prenucleosomes are formed with dNLP–histone complexes and relaxed plasmid DNA within 15 sec. Template association experiments were performed as in Figure 2C, except that the time of addition of Template 2 to the Template 1 + dNLP–histone mixture was varied as specified. (*B*) Prenucleosomes remain associated with the DNA template for at least 120 min. Template association experiments were performed as in Figure 2C, except that the time of incubation of the Template 1–histone complex with Template 2 (to allow potential exchange of histones from Template 1 to Template 2 before the addition of ACF) was varied as indicated.

neous formation of a small fraction of nucleosomes is a subject of current investigation. In this regard, it should also be noted that canonical nucleosomes are rapidly and spontaneously formed on negatively supercoiled DNA because the wrapping of the DNA around the histone octamer relieves the superhelical tension (see, e.g., Pfaffle and Jackson 1990; Nakagawa et al. 2001). Thus, negatively supercoiled DNA is "spring loaded" for nucleosome formation. In our studies of ACF-catalyzed chromatin assembly, we include purified eukaryotic topoisomerase I to maintain the DNA in a relaxed state.

- Prenucleosomes can be formed by the initial deposition of histones H3 and H4 onto the DNA followed by the subsequent addition of histones H2A and H2B. Prenucleosomes are not formed by the initial deposition of histones H2A and H2B onto the DNA.
- By atomic force microscopy, prenucleosomes are indistinguishable from nucleosomes.

The discovery of prenucleosomes additionally clarified an early paradox in chromatin assembly. By electron microscopy, it was observed that nucleosome-like structures that contain histones H2A and H3 are formed within seconds upon passage of DNA replication forks (see, e.g., McKnight and Miller 1977; McKnight et al. 1978; Sogo et al. 1986). In contrast, canonical "mature" nucleosomes, as characterized by nuclease digestion and sedimentation properties, were found to require ~10–20 min for assembly in cells (see, e.g., Seale 1975, 1976; Levy and Jakob 1978; Worcel et al. 1978; Schlaeger and Knippers 1979; Klempnauer et al. 1980; Jackson and Chalkley 1981). It was therefore wondered how could nucleosome-like particles be formed within seconds when it was also known that it takes at least 10 min to form a canonical nucleosome (see, e.g., Worcel et al. 1978). It now appears that this paradox can be explained by the rapid formation of prenucleosomes at DNA replication forks. The rapid rate of formation of prenucleosomes in vitro corresponds to the timing of the appearance of similar nucleosome-like structures (which we postulate to be prenucleosomes) at replication forks in vivo. In addition, the rate of the ACF-catalyzed conversion of prenucleosomes into canonical nucleosomes in vitro coincides with the timing of chromatin "maturation" in cells.

Hence, the initial studies revealed that prenucleosomes are rapidly formed nucleosome-like particles that do not supercoil DNA but can be converted into canonical nucleosomes with ACF or Chd1. The term "prenucleosome" was designated because these particles are precursors to nucleosomes (Fig. 1B). Moreover, as shown in Figures 2 and 3, prenucleosomes can be generated with the dNLP histone chaperone as well as with NAP1. These findings collectively suggest that prenucleosomes can be rapidly formed on newly synthesized DNA, such as during DNA replication and repair, and can remain stably associated with the DNA until they are converted into canonical nucleosomes by a motor protein.

PRENUCLEOSOMES ASSOCIATE WITH ~70–80 bp DNA

The next challenge in the study of the prenucleosome was the determination of its composition and structural organization. The first step toward this goal was achieved by the analysis of prenucleosomes by psoralen cross-linking and electron microscopy. In this method, chromatin is treated with psoralen, which cross-links the linker DNA between nucleosomes, and the DNA is visualized by electron microscopy under denaturing conditions (Sogo et al. 1986; Brown et al. 2013). The regions of DNA that are associated with histones appear as single-stranded bubbles in the electron micrographs.

When prenucleosomes and nucleosomes were compared by psoralen cross-linking and electron microscopy, it was found that nucleosomes are bound to ~140–150 bp DNA, as expected, and prenucleosomes are associated with ~70–80 bp DNA (Fig. 4; Fei et al. 2015). The association of less than one turn of DNA around the histones in a prenucleosome is the likely cause of the lack of DNA supercoiling by prenucleosomes, as seen in Torigoe et al. (2011).

The psoralen cross-linking experiments additionally allowed the comparison of the properties of prenucleosomes and nucleosomes in vitro (Fei et al. 2015) with those of chromatin particles at active and repressed promoters in vivo in yeast (Brown et al. 2013). Somewhat strikingly, the in vivo studies revealed a peak of 70–80 bp at the active *PHO5* promoter and a peak of 140–150 bp at the inactive *PHO5* promoter. Direct comparison of the in vitro and in vivo bubble size distributions suggests that active *PHO5* promoter contains prenucleosomes, whereas the inactive *PHO5* promoter contains canonical nucleosomes (Fig. 4). Thus, prenucleosomes may be present at the upstream promoter regions of active genes. This point is discussed in greater detail below.

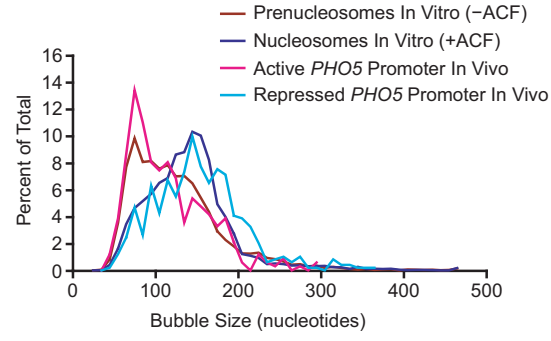

Figure 4. Prenucleosomes generated in vitro as well as noncanonical chromatin particles at the active yeast *PHO5* promoter in vivo both associate with ~70–80 bp DNA. Chromatin particles were analyzed by psoralen cross-linking followed by denaturing electron microscopy, as in Brown et al. (2013). The histone-free DNA is cross-linked by psoralen, and the resulting bubbles represent the locations of prenucleosomes and nucleosomes. The figure shows data from prenucleosomes and nucleosomes in vitro (Fei et al. 2015) and at the active versus repressed *PHO5* promoters in vivo in *Saccharomyces cerevisiae* (Brown et al. 2013). The plots show the distribution of bubble sizes as the average of 10-nt bins. (Reprinted, with permission, from Fei et al. 2015, Cold Spring Harbor Laboratory Press.)

THE PRENUCLEOSOME IS A STABLE CONFORMATIONAL ISOMER OF THE NUCLEOSOME

The observation that prenucleosomes associate with ~70–80 bp DNA led us to develop a monomeric prenucleosome (mono-prenucleosome) system. We found that prenucleosomes are formed with 80 bp DNA fragments and the four core histones under a variety of conditions that include deposition by NAP1 or dNLP as well as salt dialysis methodology (Fig. 5A; Fei et al. 2015). The formation of prenucleosomes by salt dialysis of the four core histones and an 80 bp DNA fragment suggests that the prenucleosome is the thermodynamically most stable arrangement of a histone octamer and 80 bp DNA. Additional experiments, which are described in Fei et al. (2015), further indicated that prenucleosomes contain a histone octamer rather than a histone hexamer (with two copies each of H3 and H4 and one copy each of H2A and H2B).

It was important to test, however, whether monoprenucleosomes that are assembled with 80 bp DNA can be converted into canonical nucleosomes. To address this question, we ligated DNA fragments to the 80 bp mono-prenucleosomal DNA to give mono-prenucleosomes with 85 bp of free flanking DNA on each side (Fig. 5B). We then observed that the resulting species can be assembled into a canonical nucleosome by the ACF motor protein and ATP (Fig. 5B). Thus, the mono-prenucleosomes that are formed with 80 bp DNA are functionally active.

It is intriguing that mono-prenucleosomes with free flanking DNA do not spontaneously fold into nucleosomes. This phenomenon has been observed with mono-prenucleosomes (Fig. 5) as well as with prenucleosomes on relaxed plasmid DNA (Torigoe et al. 2011). Because of the association of only ~80 bp DNA with the histones in a prenucleosome, we hypothesize that the histone octamer in a prenucleosome is slightly expanded or unfolded relative to the octamer in a canonical nucleosome. The mechanism by which ACF can convert prenucleosomes into nucleosomes remains to be determined.

Additional studies revealed that the DNA associated with the prenucleosome is at a location that is analogous to the central 80 bp in a canonical nucleosome (Fei et al. 2015). In addition, micrococcal nuclease (MNase) digestion analysis of prenucleosomes revealed that ~80 bp of DNA are protected from digestion (Fei et al. 2015). The latter observation is consistent with the psoralen cross-linking and electron microscopy data (Fig. 4) and can also be seen at low resolution in Figure 5B.

The assembly and analysis of mono-prenucleosomes has revealed that the prenucleosome is a stable conformational isomer of the nucleosome. It is associated with ~80 bp of DNA and can be converted into a canonical nucleosome by an ATP-driven chromatin assembly factor such as ACF or Chd1. In addition, we found that histone H3K56 can be acetylated by p300 in a prenucleosome but not in a canonical nucleosome (Fei et al. 2015). H3K56 is located at the DNA entry and exit points of the nucleosome. Hence, the accessibility of the p300 acetyltransferase to H3K56 in a prenucleosome is consistent with the experimentally determined central location of the 80 bp of DNA in the prenucleosome. These findings are summarized in Figure 6.

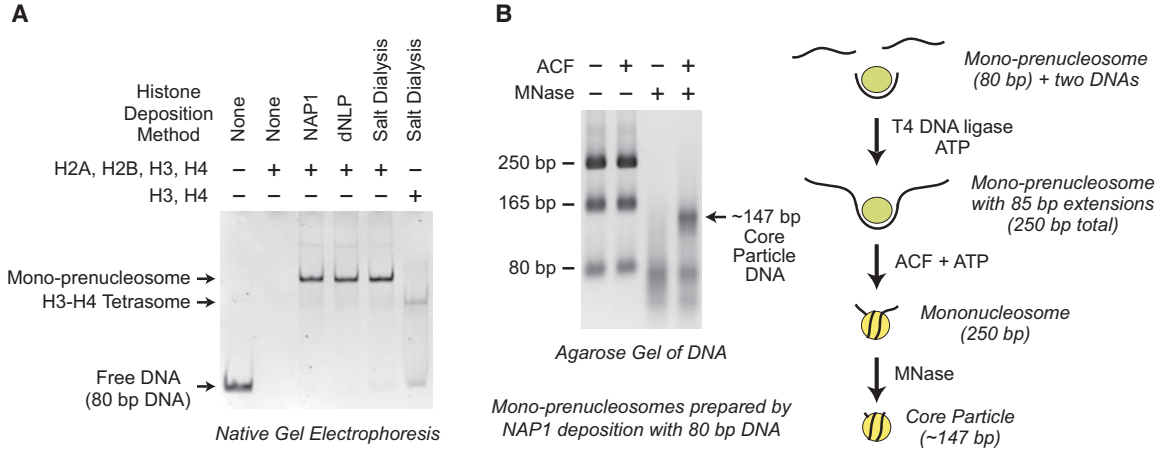

Figure 5. Assembly and analysis of monomeric prenucleosomes (mono-prenucleosomes). (*A*) Mono-prenucleosomes appear to be the thermodynamically most stable arrangement of the four core histones and 80 bp DNA at 50 mM NaCl. Mono-prenucleosomes were formed with the NAP1 or dNLP histone chaperones as well as by salt dialysis of the four core histones with an 80 bp DNA fragment. For comparison, H3-H4 mono-tetrasomes were also generated in parallel by salt dialysis with H3-H4. The histones were used at an octamer equivalent:DNA ratio of 1.0. (*B*) Prenucleosomes can be converted into canonical nucleosomes by ACF. ACF-dependent assembly of mono-prenucleosomes to canonical nucleosomes. Mono-prenucleosomes formed on 80 bp of DNA were ligated to two free 80 bp DNA fragments (each containing a single 5-nt overhang) to give mono-prenucleosomes that are flanked by 85 bp DNA extensions, as illustrated in the diagram. The resulting mono-prenucleosomes were assembled into nucleosomes by ACF. The formation of canonical nucleosomes was assessed by MNase digestion into core particles that contain ~147 bp DNA. The 80 bp and 165 bp DNA fragments are incomplete ligation products. (Reprinted, with permission, from Fei et al. 2015, Cold Spring Harbor Laboratory Press.)

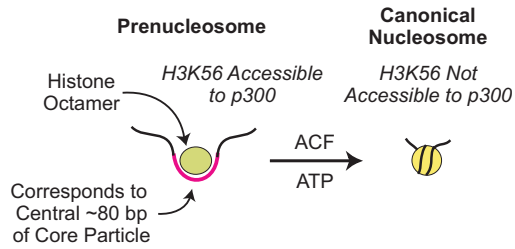

Figure 6. The prenucleosome is a conformational isomer of the nucleosome. Prenucleosomes comprise a core histone octamer and 80 bp DNA at a location that is analogous to that of the central 80 bp of the core particle. H3K56 is accessible to p300 in a prenucleosome but not in a nucleosome. Prenucleosomes can be converted into canonical nucleosomes by a motor protein such as ACF or Chd1. (Reprinted, with permission, from Fei et al. 2015, Cold Spring Harbor Laboratory Press.)

MPE-seq REVEALS PRENUCLEOSOME-LIKE PARTICLES AT THE UPSTREAM REGION OF ACTIVE PROMOTERS

To gain a different perspective on chromatin structure, we used the chemical reagent methidiumpropyl-EDTA-Fe(II) (MPE-Fe(II)) to cleave chromatin in nuclei, and then we analyzed the resulting DNA fragments by genome-wide paired-end sequencing (Ishii et al. 2015). This method, termed MPE-seq, revealed that the upstream promoter regions of active genes in mouse cells contain chromatin particles with subnucleosome-sized DNA fragments.

Because prenucleosomes associate with ~80 bp DNA, we specifically analyzed the properties of chromatin particles with 61–100 bp DNA fragments. We compared the properties of chromatin that was cleaved with MPE-Fe(II) (MPE-seq), the standard concentration of MNase (MNase-seq), or a low concentration of MNase (MNase Low-seq). As shown in Figure 7A (left panels), there is a strong peak of prenucleosome-sized (61–100 bp) DNA fragments at the upstream promoter region by MPE-seq as well as by MNase Low-seq. This location immediately upstream of the transcription start site of active promoters is often termed the "nucleosome-depleted region" (NDR) or "nucleosome-free region" (NFR). Importantly, chromatin immunoprecipitation (ChIP) data indicated that the 61–100 bp DNA particles contain histones H2B and H3 (Fig. 7A, right panels). Heat maps of MPE-seq, MNase-seq, and MNase Low-seq data with 61–100 bp DNA fragments further revealed that intensity of the MPE-seq and MNase Low-seq signals at the NDR increases with promoter activity, as assessed by RNA-seq (Fig. 7B). In addition, the MPE-ChIP-seq and MNase Low-ChIP-seq data with 61–100 bp fragments show that the strength of the H2B and H3 ChIP signals at the NDR increases with promoter activity (Fig. 7C,D). These findings suggest that there are prenucleosome-like particles at the NDRs of active promoters.

It is also notable that 61–100 bp particles are not seen in the NDRs of active genes by standard MNase-seq (Fig. 7B). The MNase sensitivity of these particles is similar to that of "fragile nucleosomes," which have been studied in yeast (see, e.g., Weiner et al. 2010; Xi et al. 2011; Knight et al. 2014; Kubic et al. 2015; also see Henikoff et al. 2011). Hence, there may be a relation between fragile nucleosomes in yeast and the prenucleosome-like particles at the NDRs of mouse cells (Fig. 7).

SUMMARY AND PERSPECTIVES

The prenucleosome is a stable conformational isomer of the nucleosome. It is associated with ~80 bp DNA and can be assembled into a canonical nucleosome by an ATP-driven chromatin assembly factor such as ACF (Fig. 6). Multiple independent lines of evidence suggest that prenucleosomes or prenucleosome-like particles are at active chromatin such as enhancers and promoters (Fig. 8A). First, there is a strong correlation between the size distributions of psoralen cross-linked bubbles in prenucleosomes assembled in vitro and the active yeast *PHO5* promoter in vivo (Fig. 5; Fei et al. 2015). Second, prenucleosome-sized DNA-containing particles with core histones are observed at the NDRs of active promoters in mouse cells (Fig. 7; also see Ishii et al. 2015). Moreover, the occurrence of these particles correlates with the level of promoter activity. Third, prenucleosomes, but not nucleosomes, can be acetylated at H3K56 by p300 (Fei et al. 2015). H3K56 acetylation is associated with chromatin assembly (see, e.g., Masumoto et al. 2005; Han et al. 2007; Chen et al. 2008; Li et al. 2008). In addition, H3K56 is acetylated at active promoters and enhancers in yeast, *Drosophila*, and humans (see, e.g., Lo et al. 2011; Venkatesh et al. 2012; Skalska et al. 2015). H3K56 acetylation in chromatin may be a marker for the presence of prenucleosomes. From a more general perspective, prenucleosomes or prenucleosome-like particles might be found at sites of DNA synthesis and/or chromatin disruption—in other words, at dynamic chromatin (Fig. 8B).

Last, an important question that remains to be resolved is whether prenucleosomes have specific functions at dynamic chromatin. It might be imagined, for instance, that the transcriptional machinery is optimized to function in conjunction with a prenucleosome. In the future, it will be interesting and important to investigate the roles of prenucleosomes at active chromatin.

ACKNOWLEDGMENTS

We thank George Kassavetis and Bing Ren for critical reading of the manuscript. We would like to express our appreciation to Christopher R. Brown, Hinrich Boeger, Sharon E. Torigoe, and Bing Ren for their stimulating and enjoyable collaborations on the psoralen cross-linking and electron microscopy (C.R.B., H.B., and S.E.T.; Fei et al. 2015) and MPE-seq (B.R.; Ishii et al. 2015) experiments. This work was supported by the National Institutes of Health (grants R01 GM041249 and R01 GM058272 to J.T.K.).

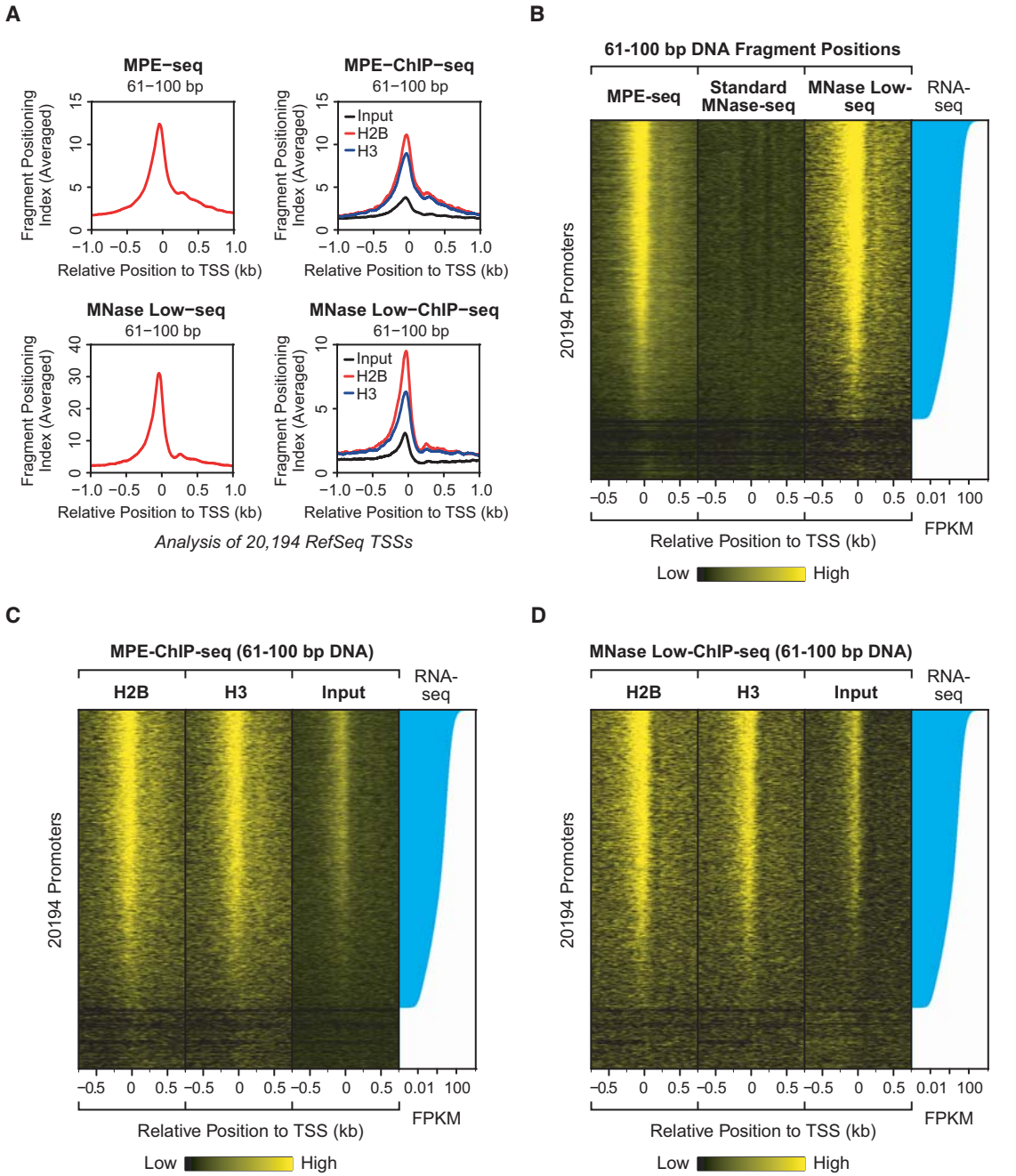

Figure 7. Prenucleosome-sized (61–100 bp) DNA-containing particles with core histones are immediately upstream of transcription start sites of active promoters in mouse J1 embryonic stem cells. The 61–100 bp data from Ishii et al. (2015) were subjected to fragment positioning analysis as described in Ishii et al. (2015). The fragment positioning index depicts the location of the central regions of the DNA fragments, which were identified by paired-end sequencing. Data from the digestion of chromatin with MPE-Fe(II), the standard concentration of MNase, or a low concentration of MNase (MNase Low) are shown. The 20,194 promoters in the heatmaps were ranked according to their transcript levels (FPKM, fragments per kilobase of exon per million fragments mapped) from RNA-seq data. (*A*) Averaged Fragment Positioning Index of chromatin digested with MPE-Fe(II) or MNase Low conditions. The *left* panels show the total 61–100 bp DNA fragments. The *right* panels show the results from ChIP analyses of soluble chromatin fragments. (*B*) Comparison of MPE-seq, standard MNase-seq, and MNase Low-seq data. Heatmaps of the fragment positioning index of 61–100 bp DNA-containing particles are shown. (*C*) Heatmaps of the MPE-ChIP-seq signal of histones H2B and H3 from 61–100 bp DNA-containing particles. (*D*) Heatmaps of the MNase Low-ChIP-seq signal of histones H2B and H3 from 61–100 bp DNA-containing particles.

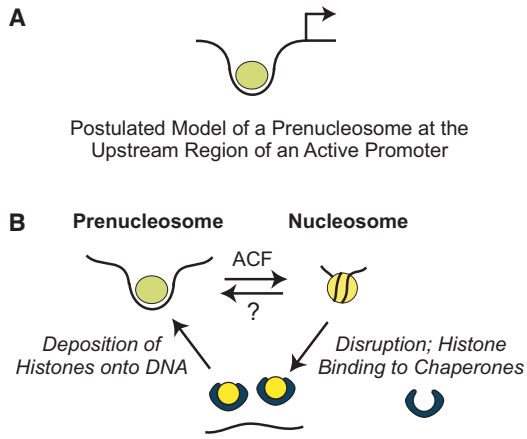

Figure 8. Postulated role of prenucleosomes in active chromatin. (*A*) Prenucleosomes or prenucleosome-related particles may be present in the upstream region of active promoters. (*B*) Model for the productive dynamic interconversion between prenucleosomes and nucleosomes. Prenucleosomes can be formed by the deposition of histones onto DNA and converted into nucleosomes by an ATP-driven motor protein such as ACF or Chd1. Nucleosomes can be disrupted by the action of enzymes such as polymerases as well as some ATP-driven chromatin remodeling factors. The resulting free histones are bound by the chaperones and then re-assembled into prenucleosomes. It is not known whether a canonical nucleosome can be directly converted into a prenucleosome. (Reprinted, with permission, from Fei et al. 2015, Cold Spring Harbor Laboratory Press.)

REFERENCES

Brown CR, Mao C, Falkovskaia E, Jurica MS, Boeger H. 2013. Linking stochastic fluctuations in chromatin structure and gene expression. *PLoS Biol* **11:** e1001621.

Chen CC, Carson JJ, Feser J, Tamburini B, Zabaronick S, Linger J, Tyler JK. 2008. Acetylated lysine 56 on histone H3 drives chromatin assembly after repair and signals for the completion of repair. *Cell* **134:** 231–243.

Fei J, Torigoe SE, Brown CR, Khuong MT, Kassavetis GA, Boeger H, Kadonaga JT. 2015. The prenucleosome, a stable conformational isomer of the nucleosome. *Genes Dev* **29:** 2563–2575.

Fyodorov DV, Kadonaga JT. 2003. Chromatin assembly in vitro with purified recombinant ACF and NAP-1. *Methods Enzymol* **371:** 499–515.

Han J, Zhou H, Horazdovsky B, Zhang K, Xu RM, Zhang Z. 2007. Rtt109 acetylates histone H3 lysine 56 and functions in DNA replication. *Science* **315:** 653–655.

Henikoff JG, Belsky JA, Krassovsky K, MacAlpine DM, Henikoff S. 2011. Epigenome characterization at single base-pair resolution. *Proc Natl Acad Sci* **108:** 18318–18323.

Ishii H, Kadonaga JT, Ren B. 2015. MPE-seq, a new method for the genome-wide analysis of chromatin structure. *Proc Natl Acad Sci* **112:** E3457–E3465.

Ito T, Tyler JK, Bulger M, Kobayashi R, Kadonaga JT. 1996. ATP-facilitated chromatin assembly with a nucleoplasmin-like protein from *Drosophila melanogaster*. *J Biol Chem* **271:** 25041–25048.

Jackson V, Chalkley R. 1981. A new method for the isolation of replicative chromatin: Selective deposition of histone on both new and old DNA. *Cell* **23:** 121–134.

Klempnauer KH, Fanning E, Otto B, Knippers R. 1980. Maturation of newly replicated chromatin of simian virus 40 and its host cell. *J Mol Biol* **136:** 359–374.

Knight B, Kubik S, Ghosh B, Bruzzone MJ, Geertz M, Martin V, Dénervaud N, Jacquet P, Ozkan B, Rougemont J, et al. 2014. Two distinct promoter architectures centered on dynamic nucleosomes control ribosomal protein gene transcription. *Genes Dev* **28:** 1695–1709.

Kubic S, Bruzzone MJ, Jacquet P, Falcone JL, Rougemont J, Shore D. 2015. Nucleosome stability distinguishes two different promoter types at all protein-coding genes in yeast. *Mol Cell* **60:** 422–434.

Levy A, Jakob KM. 1978. Nascent DNA in nucleosome-like structures from chromatin. *Cell* **14:** 259–267.

Li Q, Zhou H, Wurtele H, Davies B, Horazdovsky B, Verreault A, Zhang Z. 2008. Acetylation of histone H3 lysine 56 regulates replication-coupled nucleosome assembly. *Cell* **134:** 244–255.

Lo KA, Bauchmann MK, Baumann AP, Donahue CJ, Thiede MA, Hayes LS, des Etages SA, Fraenkel E. 2011. Genome-wide profiling of H3K56 acetylation and transcription factor binding sites in human adipocytes. *PLoS One* **6:** e19778.

Masumoto H, Hawke D, Kobayashi R, Verreault A. 2005. A role for cell-cycle-regulated histone H3 lysine 56 acetylation in the DNA damage response. *Nature* **436:** 294–298.

McKnight SL, Miller OL Jr. 1977. Electron microscopic analysis of chromatin replication in the cellular blastoderm *Drosophila melanogaster* embryo. *Cell* **12:** 795–804.

McKnight SL, Bustin M, Miller OL Jr. 1978. Electron microscopic analysis of chromosome metabolism in the *Drosophila melanogaster* embryo. *Cold Spring Harb Symp Quant Biol* **42:** 741–754.

Nakagawa T, Bulger M, Muramatsu M, Ito T. 2001. Multistep chromatin assembly on supercoiled plasmid DNA by nucleosome assembly protein-1 and ATP-utilizing chromatin assembly and remodeling factor. *J Biol Chem* **276:** 27384–27391.

Pfaffle P, Jackson V. 1990. Studies on rates of nucleosome formation with DNA under stress. *J Biol Chem* **265:** 16821–16829.

Schlaeger EJ, Knippers R. 1979. DNA-histone interaction in the vicinity of replication points. *Nucleic Acids Res* **6:** 645–656.

Seale RL. 1975. Assembly of DNA and protein during replication in HeLa cells. *Nature* **255:** 247–249.

Seale RL. 1976. Studies on the mode of segregation of histone nu bodies during replication in HeLa cells. *Cell* **9:** 423–429.

Skalska L, Stojnic R, Li J, Fischer B, Cerda-Moya G, Sakai H, Tajbakhsh S, Russell S, Adryan B, Bray SJ. 2015. Chromatin signatures at Notch-regulated enhancers reveal large-scale changes in H3K56ac upon activation. *EMBO J* **34:** 1889–1904.

Sogo JM, Stahl H, Koller T, Knippers R. 1986. Structure of replicating simian virus 40 minichromosomes. The replication fork, core histone segregation and terminal structures. *J Mol Biol* **189:** 189–204.

Torigoe SE, Urwin DL, Ishii H, Smith DE, Kadonaga JT. 2011. Identification of a rapidly formed nonnucleosomal histone-DNA intermediate that is converted into chromatin by ACF. *Mol Cell* **43:** 638–648.

Torigoe SE, Patel A, Khuong MT, Bowman GD, Kadonaga JT. 2013. ATP-dependent chromatin assembly is functionally distinct from chromatin remodeling. *Elife* **2:** e00863.

Venkatesh S, Smolle M, Li H, Gogol MM, Saint M, Kumar S, Natarajan K, Workman JL. 2012. Set2 methylation of histone H3 lysine 36 suppresses histone exchange on transcribed genes. *Nature* **489:** 452–455.

Weiner A, Hughes A, Yassour M, Rando OJ, Friedman N. 2010. High-resolution nucleosome mapping reveals transcription-dependent promoter packaging. *Genome Res* **20:** 90–100.

Worcel A, Han S, Wong ML. 1978. Assembly of newly replication chromatin. *Cell* **15:** 969–977.

Xi Y, Yao J, Chen R, Li W, He X. 2011. Nucleosome fragility reveals novel functional states of chromatin and poises genes for activation. *Genome Res* **21:** 718–724.

Cold Spring Harbor Symposia on Quantitative Biology

Prenucleosomes and Active Chromatin

Mai T. Khuong, Jia Fei, Haruhiko Ishii, et al.

Cold Spring Harb Symp Quant Biol published online January 14, 2016
Access the most recent version at doi:10.1101/sqb.2015.80.027300