Multistep Chromatin Assembly on Supercoiled Plasmid DNA by Nucleosome Assembly Protein-1 and ATP-utilizing Chromatin Assembly and Remodeling Factor

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We examine in vitro nucleosome assembly by nucleosome assembly protein-1 (NAP-1) and ATP-utilizing chromatin assembly and remodeling factor (ACF). In contrast to previous studies that used relaxed, circular plasmids as templates, we have found that negatively supercoiled templates reveal the distinct roles of NAP-1 and ACF in histone deposition and the formation of an ordered nucleosomal array. NAP-1 can efficiently deposit histones onto supercoiled plasmids. Furthermore, NAP-1 exhibits a greater affinity for histones H2A-H2B than does naked DNA, but in the presence of H3-H4, H2A-H2B are transferred from NAP-1 to the plasmid templates. These observations underscore the importance of a high affinity between H2A-H2B and NAP-1 for ordered transfer of core histones onto DNA. In addition, recombinant ACF composed of imitation switch and Acf1 can extend closely packed nucleosomes, which suggests that recombinant ACF can mobilize nucleosomes. In the assembly reaction with a supercoiled template, ACF need not be added simultaneously with NAP-1. Regularly spaced nucleosomes are generated even when recombinant ACF is added after core histones are transferred completely onto the DNA. Atomic force microscopy, however, suggests that NAP-1 alone fails to accomplish the formation of fine nucleosomal core particles, which are only formed in the presence of ACF. These results suggest a model for the ordered deposition of histones and the arrangement of nucleosomes during chromatin assembly in vivo.

The assembly of genomic DNA and histones into chromatin is a fundamental process of eukaryotes that affects a broad range of biological phenomena, including DNA replication, DNA repair, gene expression, and progression through the cell cycle (for reviews, see Refs. 1–7). The basic structural unit of chromatin is the nucleosome, consisting of 145 base pairs (bp)1 of DNA wrapped around an octamer of the core histones H2A, H2B, H3, and H4. Crystallographic studies have shown that an H3-H4 tetramer occupies the central region of the nucleosome, and the H2A-H2B dimers bind to the peripheral region (8, 9). Thus, it is implicit in the structure of the nucleosome that H3 and H4 must be deposited prior to the incorporation of H2A and H2B. In agreement with this, several studies of histone transfer and of chromatin assembly during DNA replication have indicated that H3-H4 deposition occurs first, then H2A-H2B, followed by linker histones (such as histone H1) and the establishment of the ordered nucleosomal arrays that are characteristic of bulk chromatin in vivo (for reviews, see Refs. 10–13).

Biochemical studies have led to the identification of proteins that mediate the reconstitution of core histones into nucleosomes. Nearly all of these factors are core histone-binding proteins that contain stretches of acidic amino acid residues. Some of these histone chaperones, such as nucleoplasm and nucleosome assembly protein-1 (NAP-1), exhibit a preference for binding to histones H2A and H2B relative to histones H3 and H4. Other histone chaperones, including replication-coupling assembly factor, chromatin assembly factor-1, N1/N2, and Sp6, associate preferentially with H3 and H4 (for reviews, see Refs. 10–14). It has also been observed that newly synthesized histones are acetylated (such as at positions 5 and 12 of histone H4) and then subsequently deacetylated after assembly into chromatin (for reviews, see Refs. 15 and 16). Thus, factors that mediate histone acetylation or deacetylation may participate in the chromatin assembly process indirectly by the covalent modification of histones. Among H3 and H4 chaperones, Drosophila anti-silencing function-1 and chromatin assembly factor-1 were shown to be associated with newly synthesized, acetylated core histones (17, 18). To varying extents, these core histone chaperones can mediate the deposition of histones onto DNA via an ATP-independent process that yields chromatin consisting of randomly distributed nucleosomes. A similar distribution of nucleosomes can be accomplished by the method of salt gradient dialysis or by using polyamions, such as polyglutamate or RNA, which can interact with the histones and prevent their nonspecific aggregation with DNA. This random deposition process, however, is generally inefficient and does not yield periodic arrays of nucleosomes that resemble those seen in native chromatin (for reviews, see Refs. 10, 12, and 13).

The assembly of regularly spaced nucleosome arrays is an ATP-dependent process, as was initially observed in an extract derived from Xenopus oocytes (19). In studies of chromatin assembly factors from Drosophila embryos, the ATP-utilizing component of the chromatin assembly machinery (termed ACF for ATP-utilizing chromatin assembly and remodeling factor) was identified, and its subunit Acf1 was cloned (20, 21). Recombinant ACF consisting of dAcf1 and Drosophila imitation

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1 The abbreviations used are: bp, base pair(s); NAP-1, nucleosome assembly protein-1; ACF, ATP-utilizing chromatin assembly and remodeling factor; dNAP-1, Drosophila nucleosome assembly protein-1.
**Fig. 1.** The affinity between H2A-H2B and NAP-1 is higher than that between H2A-H2B and DNA. After incubation of core histones with NAP-1 and DNA, the histones were separated by density gradient sedimentation. Fractions from the gradient were analyzed using 12% SDS gel electrophoresis to detect core histones and NAP-1 and 1% agarose gel electrophoresis to detect DNA. Because free core histones were sedimented in the top lane (data not shown), core histones H2A-H2B were sedimented together with NAP-1 in lanes 2 and 3 of A and B, indicating that they bound to NAP-1 either in the absence or in the presence of DNA. In the absence of NAP-1, H2A-H2B bound to DNA (A, lanes 6 and 7). Core histones H3-H4 bound to NAP-1 in the absence of DNA (D, lanes 3 and 4) but were transferred to DNA when DNA was added (E, lanes 5–7). When H3-H4 were incubated with DNA in the absence of NAP-1, they sedimented at the bottom of the gradient, possibly due to aggregation (F, lane 11).

### MATERIALS AND METHODS

**Purification of Recombinant Proteins**—Recombinant proteins and ACF were prepared essentially as described previously (21). His-tagged *Drosophila* NAP-1 was purified from baculo-virus-infected Sf9 cells by nickel-nitrilotriacetic acid affinity chromatography (Operon Technologies, Inc., Alameda, CA) affinity chromatography, followed by three additional chromatographic steps: Q-Sepharose FF, SP-Sepharose FF, and Q-Sepharose FF (all media were from Amersham Pharmacia Biotech). Peak fractions of the final Q-Sepharose FF column were pooled and used in the assays.

**Chromatin Assembly Reactions**—Chromatin assembly reactions were performed essentially as described by Bulger et al. (22) and Itou et al. (23) using supercoiled plasmid DNA. A standard reaction contained plasmid DNA (0.4 μg), purified core histones from *Drosophila embryos* (0.35 μg), purified recombinant dNAP-1, purified recombinant ACF, ATP (3 mM), and an ATP-regenerating system (30 mM phosphocreatine and 1 μg/ml creatine phosphokinase). All experiments were done a minimum of three times to ensure the accuracy of our results.

**Density Gradient Analysis of Chromatin**—After chromatin was assembled, reaction mixtures were subjected to 15–40% glycerol density gradient sedimentation in an SW60 (Beckman Coulter, Inc., Fullerton, CA) rotor at 60,000 rpm for 2.5 h at 4 °C. Part of the gradient fractions were digested with proteinase K, deproteinized with phenol-chloroform, and subjected to 1% agarose gel electrophoresis followed by ethidium bromide staining. The remaining portions of the samples were trichloroacetic acid-precipitated and analyzed by 12% SDS-polyacrylamide gel electrophoresis followed by Coomassie Brilliant Blue R-250 staining.

**Sample Preparation for Atomic Force Microscopy Imaging**—Chromatin was assembled as described above, except with 7 mM KCl instead of 50 mM KCl; reaction mixtures were then subjected to 15–40% glycerol density gradient sedimentation. Part of the fraction was analyzed by 1% agarose gel electrophoresis. The peak fraction was fixed with 0.1% glutaraldehyde at 4 °C for 10 h and subsequently dialyzed against 7 mM KCl HE (25 mM Hepes and 0.1 mM EDTA) for 10 h. Samples were applied on a 1 nm spermidine-treated mica surface. After 5 min the mica was gently washed with water and dried under nitrogen gas. Imaging was done with an SPA400 SP3800N probe station (Seiko Instruments, Inc., Matsudo, Japan) in air under cyclic mode at room temperature using a cantilever with a spring constant of 40 N/m (Seiko Instruments, Inc., Matsudo, Japan).

### RESULTS AND DISCUSSION

**Stepwise Core Histones Transfer onto Supercoiled Plasmid DNA**—Several in vivo and in vitro experiments have proven that chromatin assembly is a staged process. Worcel et al. (24) have shown that newly synthesized DNA is enriched with newly synthesized histones H3 and H4, whereas newly synthesized H2A and H2B associate with chromatin that has properties similar to those of bulk nonreplicating chromatin. Worcel et al. (24) concluded that newly synthesized histones associate with newly synthesized DNA in a sequential order; histones H3 and H4 are deposited first, and then histones H2A and H2B are deposited (for reviews, see Refs. 10, 12, and 13). From crystallographic studies of the structure of the nucleosome, it is clear that histones H3 and H4 need to be deposited prior to the incorporation of H2A and H2B. It is also well characterized that the histone octamer is unstable under physiological ionic conditions in the absence of DNA, dissociating into the H3-H4 tetramer and two H2A-H2B dimers (for reviews, see Refs. 10 and 13). However, it is still yet to be clarified how the chromatin assembly machinery ensures this ordered deposition of core histones.

To understand the mechanisms of coordinated histone deposition in vivo, we attempted to decipher how histones are transferred onto DNA in vitro. ACF and NAP-1 can assemble nucleosomes from different templates, such as relaxed, supercoiled, and linearized plasmid DNA in vitro (20). When the plasmid is in a relaxed state, in addition to NAP-1, ACF is essential for efficient nucleosome formation (20, 22, 23). Pfaffle and Jackson (25) showed, however, that if the plasmid is negatively supercoiled, nucleosome transfer occurs very rapidly even when using polyglutamic acid as a histone chaperone. In this study, we used supercoiled plasmid, which facilitated core histone transfer only via a histone chaperone.

The manner by which core histones bind to NAP-1 and DNA...
demonstrates how stepwise transfer occurs. NAP-1 was originally identified in mammalian cells as a core histone-binding protein that facilitates random, ATP-independent nucleosome deposition (26, 27), as monitored by the supercoil of DNA that arises upon formation of the nucleosomes (28). In the reaction that mediates the ATP-facilitated assembly of regularly spaced nucleosomal arrays in chromatin, we have shown that NAP-1 works with ACF. NAP-1 binds to histones H2A and H2B in vivo (23, 29) as well as in vitro (23, 30). The comparison of the affinity between NAP-1 and core histones H2A-H2B and that between DNA and H2A-H2B indicates an important role of NAP-1 in this stepwise transfer (Fig. 1, A–C). Core histones H2A-H2B bound to NAP-1 in the absence of H3-H4 (A, lanes 2 and 3). When increasing amounts of H3-H4 were added, H2A-H2B gradually transferred to DNA in proportion with H3-H4 (B, lanes 4–6; C, lanes 5–7). All the H2A-H2B histones were transferred to DNA when stoichiometric amounts of H3-H4 were added (D, lanes 5–8).

Based on the observations of Pfaffle and Jackson (25), when all four core histones are added, they apparently go onto the supercoiled plasmid DNA. Thus, we examined what happens when different amounts of H3-H4 are added to a reaction consisting of H2A-H2B, NAP-1, and DNA (Fig. 2). The H2A-H2B histones were transferred to DNA in proportion with H3-H4 (Fig. 2B, lanes 4–6; C, lanes 5–7). All the H2A-H2B histones were transferred to DNA when stoichiometric amounts of H3-H4 were added (D, lanes 5–8).

**Fig. 2.** The recruitment of H2A-H2B from NAP-1 to the subnucleosome due to a change of affinity. After incubating core histones H2A-H2B and H3-H4 with NAP-1 and DNA at the different ratios indicated, the histones were separated by density gradient sedimentation to decipher any interactions. Core histones H2A-H2B bound to NAP-1 in the absence of H3-H4 (A, lanes 2 and 3). When increasing amounts of H3-H4 were added, H2A-H2B gradually transferred to DNA in proportion with H3-H4 (B, lanes 4–6; C, lanes 5–7). All the H2A-H2B histones were transferred to DNA when stoichiometric amounts of H3-H4 were added (D, lanes 5–8).

Multistep Chromatin Assembly
H2B bound to NAP-1 even in the presence of DNA (see Figs. 1, A and B and 2A). When H3-H4 was added, the H2A-H2B dimers were transferred from NAP-1 to DNA (Fig. 2, A and B). A, a mixture of core histones H3-H4 (0.5 μg) and NAP-1 (4 μg) was immunoprecipitated with anti-H2A-H2B. B, a mixture of core histones H2A-H2B (0.5 μg), H3-H4 (0.5 μg), and NAP-1 (8 μg). C, a mononucleosome made from ~1 μg of histone octamer by salt dialysis was immunoprecipitated with anti-H2A-H2B. The corresponding pre-immune serum was used as a control for each experiment. The resulting samples were washed and analyzed by 12% polyacrylamide SDS gel electrophoresis and subsequent Western blots with anti-H2A-H2B and anti-H3-H4. Sup, supernatant; IP, immunoprecipitation.

FIG. 3. A histone octamer is not generated on the NAP-1. In vitro communoprecipitation with purified anti-H2A-H2B was performed from different materials to find out where the histone octamer was generated efficiently. A, a mixture of core histones H3-H4 (0.5 μg) and NAP-1 (4 μg) was immunoprecipitated with anti-H2A-H2B. B, a mixture of core histones H2A-H2B (0.5 μg), H3-H4 (0.5 μg), and NAP-1 (8 μg). C, a mononucleosome made from ~1 μg of histone octamer by salt dialysis was immunoprecipitated with anti-H2A-H2B. The corresponding pre-immune serum was used as a control for each experiment. The resulting samples were washed and analyzed by 12% polyacrylamide SDS gel electrophoresis and subsequent Western blots with anti-H2A-H2B and anti-H3-H4. Sup, supernatant; IP, immunoprecipitation.

FIG. 4. Recombinant ACF can mobilize nucleosomes. Closely packed nucleosomes were reconstituted by salt dialysis techniques with purified core histones and plasmid DNA and were enriched by sucrose gradient sedimentation. The resulting chromatin (consisting of about 0.5 μg of DNA and 0.5 μg of core histones) was incubated with ATP (3 mM; in the absence of an ATP-regenerating system). ACF (~3 units) was added in reactions 2, 4, and 6 (+). At 30 min and 1 and 2 h after starting the reaction, the samples were subjected to micrococcal nuclease digestion with two different amounts of enzymes. The molecular mass markers were 123-bp ladders (Life Technologies, Inc.). The positions of the DNA fragments that correspond to pentanucleosomes are depicted by open circles without ACF (−; reactions 1, 3, and 5) and by open stars with ACF (+; reactions 2, 4, and 6). ACF gradually modulated the internucleosomal spacing of chromatin.
the nucleus during early S phase but not during the G2 phase (23). However, staining reveals the presence of a small amount of NAP-1 in the nucleus at all stages of the cell cycle. In addition to NAP-1 being a chaperone for core histones, these data support the idea that NAP-1 plays a role in the fine-tuning of chromatin assembly and disassembly coupled with transcriptional activation. Kellogg and Murray (34) reported that in yeast the deletion of NAP-1 in the wild-type strain has no obvious effect on cell growth; however, the cells of the Dnap-1 strain are often found in clumps of connected cells with elongated shapes. There might be functional redundancy between NAP-1 and another NAP-1-related protein, SET, in Drosophila and in humans (35, 36), and it remains to be extensively investigated whether there are any fine changes in the chromatin structure or transcriptional regulation associated with the function of NAP-1 in vivo.

**Histone Octamer Is Made after Histone Transfer**—To decipher the timing of histone octamer formation, we performed a coimmunoprecipitation with anti-H2A-H2B antibody (Fig. 3). Core histones H3-H4 were not precipitated with anti-H2A-H2B. When NAP-1 and a stoichiometric amount of H2A-H2B were added, however, half of the H3-H4 precipitated. By using mononucleosomes made by salt dialysis, all H3 and H4 histones were precipitated with H2A and H2B. That is, if there is a histone octamer, almost all H3 and H4 are coimmunoprecipitated with H2A and H2B. Because NAP-1 appears to be multimeric, its acidic region can simultaneously associate with both H2A-H2B dimer and H3-H4 tetramer even if a histone octamer is not made. Using crude *Drosophila* extract, we also found that anti-NAP-1 coimmunoprecipitated H2A and H2B but not H3 and H4 (23). Thus, it is reasonable to conclude that a histone octamer does not form efficiently on NAP-1 in solution and that the formation of a histone octamer in vitro is a stepwise, ordered process including: 1) the transfer of H3-H4 onto DNA and 2) the recruitment of H2A-H2B from NAP-1 to the subnucleosome due to a change of affinity.

**Nucleosome Is Mobilized by Recombinant ACF**—In a previous study we have shown that native ACF can extend a closely packed nucleosomal repeat (~146 bp) made by salt dialysis to a more physiological repeat length of ~160 bp (20). We tested whether recombinant ACF comprised of imitation switch and Acf1 also has this property. Chromatin that is closely packed (i.e. not evenly spaced) made by dialysis from 1.0 M to 50 m M KCl was used in this reaction. To test whether this reaction occurs gradually, a micrococcocal nuclease assay was performed at different time points. Without ACF, the repeat length of the nucleosomal array was 148 bp (Fig. 4, reaction 1). Two h after

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**FIG. 5.** All core histones are transferred onto supercoiled plasmid DNA within 10 min. A nucleosome assembly reaction was performed from supercoiled plasmid template in the presence of NAP-1 and topoisomerase I with or without core histones. At different time points, the reactions were stopped, and the samples were deproteinized. The resulting DNA was subjected to 1% agarose gel electrophoresis and then visualized by staining with ethidium bromide (A and B). The nucleosome assembly reaction was started with supercooled plasmid template in the presence of NAP-1 and core histones either 10 min or 1 h before applying glycerol gradient sedimentation. The gradient fraction was analyzed by 12% polyacrylamide SDS gel electrophoresis and stained with Coomassie Brilliant Blue R-250. Part of the gradient fraction was analyzed by 1% agarose gel electrophoresis and subsequent ethidium bromide staining (C and D).

**FIG. 6.** Nucleosome transfer and sliding need not be coupled. Chromatin assembly reactions were performed with or without the following components: dNAP-1 (6 μg), DNA (0.5 μg), and core histones (0.4 μg). ACF (3 units) was added either at the same time as these components or 1, 2, or 3 h after starting the reaction. At 5 h after adding ACF, each sample was partially digested with two different concentrations of micrococcal nuclease and then deproteinized. For each reaction condition, the different lanes represent increasing concentrations (from left to right) of micrococcal nuclease used to digest the chromatin. The resulting DNA fragments were subjected to agarose gel electrophoresis and visualized by staining with ethidium bromide. The molecular mass markers were 125-bp ladders (Life Technologies, Inc.).
adding ACF, the repeat length of the nucleosomal array was 160 bp (Fig. 4, reaction 6). One h after adding ACF, the repeat length of the nucleosomal array (Fig. 4, reaction 4) was between the repeat lengths of the nucleosomal arrays incubated with ACF for 30 min and 2 h (Fig. 4, reactions 2 and 6). These results indicate that, in the presence of ACF, the repeat length extends gradually from 148 to 160 bp and that recombinant ACF can mobilize nucleosomes gradually.

The imitation switch family of remodeling complexes, such as ACF, nucleosome remodeling factor, chromatin accessibility complex, and remodeling and spacing factor, exhibit a variety of properties, including: 1) the ordering of disordered nucleosomal arrays (20, 37, 38), 2) the remodeling of local chromatin structure in the presence of a DNA-binding transcriptional activator protein (20, 38–40), 3) the induction of restriction endonuclease sensitivity on nucleosomal arrays (37, 41), 4) the stimulation of nucleosome sliding (42, 43), and 5) the assembly of free histones into nucleosomes (20, 21). We demonstrated that recombinant ACF can mobilize nucleosomes, which is similar to activities 1 and 4 listed above. It is reasonable to think that the nucleosome mobilization activity of recombinant ACF plays a role in chromatin maturation.

Recently McQuibban et al. (44) reported that yeast nucleosome assembly protein-1 can space nucleosomes without any ATP present. The major difference between chromatin made by yNAP-1 and that made by dNAP-1 and ACF is the repeat length of the nucleosomal array: 146 bp by yeast nucleosomal assembly protein versus 160 bp by dNAP-1 and ACF. This difference supports our model that ACF plays a role in chromatin maturation by mobilizing nucleosomes.

**Kinetics of Histone Transfer and Chromatin Maturation**

Based on a supercoiling assay, NAP-1 and ACF cooperatively introduced negative supercoiling into closed, relaxed, circular DNA (22, 23). The formation of one nucleosome causes a change in the linking number of about −1, and thus the number of the nucleosomes assembled on a closed circular template is approximately equal to the number of negative supercoils that remain after removal of the histones in the presence of topoisomerase I (for reviews, see Refs. 10, 12, and 13). When a supercoiled template was used, topoisomerase I added at the start of the reaction never relaxed the template (Fig. 5A). The same amount of topoisomerase I used in this experiment relaxed this plasmid DNA in 2 min; relaxation was completed within 10 min without core histones (Fig. 5B). Thus, kinetics with a supercoiled template suggested that core histone transfer by NAP-1 was completed within 10 min. This is comparable with similar experiments using polyglutamic acid (24). Consistent with the supercoiling assay, glycerol gradient sedimentation with supercoiled plasmid and recombinant NAP-1 showed that core histones were transferred from NAP-1 (Fig. 5C, lane 2) onto DNA (Fig. 5C, lanes 4, 5, and 6) within 10 min, and there was no change 60 min later (compare Fig. 5, C and D). Both the supercoiling assay and glycerol gradient sedimentation showed that core histone transfer is very rapid with a supercoiled template.

Thus, we can focus on the distinct role of NAP-1 and ACF. By itself, NAP-1 was able to transfer core histones onto supercoiled plasmid DNA, and a periodic nucleosomal array was observed when ACF was added. This evidence strongly suggested that nucleosomal maturation was mainly comprised of nucleosome mobilization by ACF. In addition, ACF did not have to be added simultaneously with NAP-1. Regularly spaced nucleosomes were generated even if ACF was added at different time points throughout the reaction (Fig. 6). The 10-min transfer reaction should have been completed by the time ACF was added 1, 2, and 3 h after starting the assembly reaction by...
NAP-1. Although NAP-1 and ACF work cooperatively in the chromatin assembly, the reactions mediated by each factor can be separated into two distinct steps; 1) core histones are transferred by NAP-1, and 2) chromatin is matured by ACF using a supercoiled template in vitro.

ACF Is Essential for Nucleosome Core Particle Formation—Although we have made progress with our biochemical studies of chromatin assembly, many structural questions remain to be answered. At low ionic strength, electron microscopy typically shows that chromatin looks like beads on a string. However, there has been concern that strong interactions of the fiber with the electron microscope support surface and the dehydration produced by the intense vacuum conditions could distort the structure. Atomic force microscopy does not present these problems. Leuba et al. (45) used tapping mode atomic force microscopy to observe chicken chromatin fibers. It is possible to observe in vitro assembled chromatin relatively easily by this technique. In addition, direct images can provide more information than just biochemical assays can. Images from tapping mode atomic force microscopy are shown in Fig. 7, A–C. By using this technique, we observed the core histone transferred onto DNA in the presence of the NAP-1. The size of each particle was irregular, and the mean size (calculated by the long axis and short axis) was 15.9 nm (n = 26; S.D. = 1.0). The mean number of particles on one plasmid was 8.7 (S.D. = 2.3; Fig. 7A). Most of the particles did not look like nucleosomes, which suggests two possibilities. First, because of the irregular spacing, more than one nucleosome may appear to be one particle. Alternatively, some sort of non-nucleosomal histone–DNA complex may be formed in the absence of ACF. Naked DNA without nucleosomal particles was often seen in the absence of ACF (indicated with arrowheads in Fig. 7A). However, with the addition of ACF before and after histone transfer, the mean size of the particle was 13.8 nm (n = 37; S.D. = 0.56) and 12.9 nm (n = 43; S.D. = 0.35), respectively, and the mean number of particles on one plasmid was 12.3 (S.D. = 2.3; Fig. 7B, C), respectively. This experiment was done more than five times to ensure the accuracy of our results, which suggest that ACF, when added either before or after histone transfer, can generate fine nucleosomal structures. These data are direct evidence that ACF plays a role in chromatin maturation, possibly through its nucleosome-mobilizing activity. Both biochemical and atomic force microscopy analyses illustrate the chromatin assembly consisting of stepwise core histone transfer by a chaperone and subsequent chromatin maturation by ACF (Fig. 8). It should be noted that these distinctly ordered steps can be separated in vitro (Fig. 8A), or may lead directly to the formation of a nucleosomal array without allowing us to witness the clumpy complexes obtained with NAP-1 and histones through the process (Fig. 8B).

Conclusions—We used a purified recombinant system to clarify the mechanisms of chromatin assembly. This biochemical approach allowed us to decipher finer aspects of the chromatin assembly process. Our results reveal the important role of the NAP-1 in the stepwise transfer of core histones and the role of ACF in chromatin maturation. This system, together with atomic force microscopy, should be a useful tool for the direct examination of detailed mechanisms of other phenomena, including chromatin remodeling coupled with transcription, repair, and recombination.

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