Recombinant yeast nucleosome assembly protein (yNAP-1) facilitates the formation of uniformly spaced nucleosomes from high molecular weight DNA and core histone octamers. No additional factors or metabolites are required. The repeat length of the chromatin produced is about 146 base pairs. To obtain the most distinct nucleosomal ladders, the core histones must pre-exist as an octamer complex. yNAP-1 forms complexes with core histones as judged by native gel electrophoresis, chemical cross-linking, limited histone proteolysis, and affinity blotting. A discrete complex was observed with a probable ratio of yNAP-1 to histone octamer of 4:1. Chromatin produced by salt dialysis does not contain uniformly spaced nucleosomes, but subsequent incubation with yNAP-1 creates uniform spacing. Trypsin-treated core octamers that lack amino termini, although capable of forming core particles with core-length DNA by salt dialysis, are not assembled by yNAP-1 into uniformly spaced nucleosomes on high molecular weight DNA. Proteolytic removal of the amino termini of the core histones precludes complex formation between a histone octamer and yNAP-1. Affinity blotting also demonstrates that yNAP-1 binds linker histones and high mobility group (HMG)-1/HMG-2 but not HMG-14. Competition experiments with poly-L-arginine, poly-L-lysine, and protamine reveal that yNAP-1 binds to core and linker histones more tightly despite the much higher positive charge densities of the former molecules. Naturally occurring acetylated histone H4 species show no evidence for differential yNAP-1 binding. yNAP-1 is not bound tightly to the resulting chromatin after deposition and thus could act catalytically.

In eukaryotes, chromosomal DNA encircles octamers of core histones resulting in arrays of nucleosomes which represent the first level of chromatin organization. To a first approximation, these nucleosomal arrays are spaced uniformly with characteristic repeat lengths that can vary from species to species, from tissue to tissue, and even with the transcriptional state of the chromatin under consideration (1). Although the primary organization of nucleosomes along the chromatin fiber appears uniform, many studies have shown convincingly that chromatin can exist in a variety of states characterized by the sensitivity of the DNA to cleavage by chemical agents and nucleases (2). These states can change markedly with gene expression and are thought to be associated with nucleosome loss, disruption, or remodeling (3, 4).

Core nucleosomes and chromatin can be reconstituted readily in vitro from their constituents by methods such as dialysis from high ionic strength solutions (2) or by the addition of negatively charged macromolecules such as nucleoplasmin, polyglutamic acid, pectin, and RNA (5–9). These protocols result in the non-uniform placement of nucleosomes along the chromatin fiber which, when digested with micrococcal nuclease, results mainly in mononucleosomes and spaceless dinucleosomes (10). Adding DNA to preparations of whole, unfractionated cellular, oocyte, or embryonic extracts (11–14) as well as partially purified fractions (15–16) supplemented with exogenous core histones and ATP will produce ordered nucleosomal arrays, thus demonstrating that soluble factors can place histones onto DNA in an organized manner with a physiological repeat length.

A variety of factors is reported to influence the nucleosomal repeat length of these in vitro assembled chromatin. These include ionic strength and DNA topology (1, 17), linker histones (1, 10), high mobility group (HMG)1 proteins (6), and assembly factors such as yeast or Drosophila nucleosome assembly protein (y or dNAP-1) (15). Furthermore, linker histones in conjunction with polyglutamic acid will under certain conditions facilitate the formation of uniformly spaced nucleosomes from core histones and plasmid DNA (18).

Recombinant yNAP-1 is capable of forming histone-DNA complexes and forming nucleosomes on plasmid DNA (19, 20). yNAP-1 as well as nucleoplasmin will act to facilitate transcription factor binding to a nucleosome-containing target sequence (21). Recently Kadonaga and co-workers (15) reported that both recombinant Drosophila and yeast NAP-1 will create a chromatin of uniformly spaced nucleosomes of a short repeat length from plasmid DNA and core histones. Furthermore they found that adding a Drosophila CAF-1 fraction and ATP to the assembly mixture results in the rapid formation of long arrays of uniformly spaced nucleosomes of a physiological repeat. Their results clearly demonstrate that although yNAP-1 is required to produce uniformly spaced nucleosomes, other proteins in the dCAF-1 fraction facilitate the assembly, both kinetically and qualitatively in terms of the resulting nucleosomal repeat.

Immunoprecipitation of human and Drosophila cytosolic extracts with anti-NAP antibodies suggests that histones 2A and 2B are the principal molecules complexed with NAP-1 in vivo (15, 22). In yeast, immunoprecipitation of yNAP-1 reveals an in vivo association with the cyclin Clb2 (23). Cell cycle-dependent changes in the subcellular distribution of yNAP-1 suggest a role in replication-dependent chromatin assembly (15, 22). Most of these effects require that yNAP-1 interact with the...
core histones.

Here we report a study of the *in vitro* ability of recombinant *Saccharomyces cerevisiae* NAP-1 to assemble core histones and high molecular weight DNA into a synthetic chromatin at physiological ionic strengths. Our results indicate that the assembled chromatin is characterized by uniformly spaced nucleosomes with a very short repeat length. The short repeat is largely independent of the underlying DNA sequence but depends upon the preexistence of histone octamers and the presence of the amino termini of the core histones. Furthermore we show that yNAP-1 is able to remodel a salt dialysis-produced chromatin into a short repeat chromatin. Because the nature of the interaction of NAP-1 with core histones has not been studied in any detail previously, an analysis of the ability of recombinant yNAP-1 to bind to the histone octamer, individual core and linker histones, as well as other basic proteins was made. Our results reveal that yNAP-1 binds specifically and reversibly to the highly positively charged disordered segments of all of the histones.

**EXPERIMENTAL PROCEDURES**

**Materials**—Chicken chromatin, poly- and mononucleosomes, and core-length DNA were prepared as described (24, 25). Chicken histone octamers were isolated from salt-stripped chicken erythrocyte chromatin (26) by the method of Simon and Felsenfeld (27) and stored at –20°C in 2 mM NaCl and 50% glycerol. Trypsin-treated chicken histone octamers were prepared by trypsin digestion of salt-stripped chromatin in 25 mM NaCl, 10 mM Tris, pH 7.5, at a mass ratio of 1:1000:1 for 5 min at 37°C followed by the addition of a 100-fold excess of soybean trypsin inhibitor and hydroxyapatite chromatography (27). Chicken core and linker histones were extracted from chicken erythrocyte nuclei with 0.4 M HCl, the core histones precipitated with 7% perchloric acid, and the spun supernatant adjusted to 20% trichloroacetic acid to precipitate the linker histones. The two precipitates were washed once with acidified acetone and dry acetone, vacuum dried, and washed [35S]cysteine (ICN, 1,000 Ci/mmol) in 100 µl of TE, pH 8, overnight at room temperature. Typically the resulting yNAP-1 had a specific activity of 1.5 Ci/mmol (7 × 10^4 dpmmg). Mono[fluorescein-labeled yNAP-1 was prepared by incubating yNAP-1 (100 µg) in 100 µl of 0.1 M NaHCO₃, pH 8, with 6 nmol of N-hydroxysulfosuccinimidyl-5-carboxyfluorescein at room temperature for 30 min. The reaction was quenched with 10 nmol of 3,3’diamino-N-methyl dipropylamine. The fluorescein-labeled yNAP-1 was precipitated by the addition of 1 ml of 20% trichloroacetic acid. The precipitated fluorescein, dry acetone, vacuum dried, and dissolved in 100 µl of TE, pH 8. The resulting yNAP-1 had an average of 1 mol of fluorescein per mol of yNAP-1 based on A₄₅₀ nm and ε₄₅₀ = 74,000. SDS-PAGE and fluorescence imaging revealed less than 25% unbound fluorescein that runs with the dye front on SDS gels. N-Hydroxysulfosuccinimidyl-5-carboxyfluorescein was synthesized by methods described elsewhere (28). The fluorescein-labeled yNAP-1 was prepared in the same way as the fluorescein-labeled yNAP-1 except N-hydroxysulfosuccinimidyl [14C]acetate was used. Less than 1 mol of acetate was incorporated per yNAP-1.

**yNAP-1-assisted Chromatin Assembly**—High molecular weight chicken genomic DNA (40 µl of 0.5 mg/ml in TE) was mixed with yNAP-1/histone mixtures (125 µl of 300,150 µg/ml, respectively, pre-mixed for at least 20 min) at physiological ionic strength (150 mM NaCl, 25 mM Tris, 1 mM EDTA, 10 mM dithiothreitol, pH 8), incubated for 3 h at 37°C, and then subjected to micrococcal nuclease digestion (Sigma, 0.75 unit/µg of DNA) for various times at 37°C after adding CaCl₂ to 5 mM. After deproteinization the DNA was resolved by 1.5% agarose electrophoresis and subsequently stained with ethidium bromide. In experiments with [35S]-labeled DNA, a Molecular Dynamics PhosphorImager was used for visualization of the DNA.

**Salt Dialysis Chromatin and Nucleosome Assembly**—Chromatins were prepared from high molecular weight DNA and plain and trypsin-treated histone octamers according to Stein (10) by dialysis from 1 M NaCl. Core nucleosomes were reconstituted from histones and core length 146-bp DNA as described (29).

**Chemical Cross-linking**—Histone-yNAP-1 complexes were cross-linked with disuccinimidyl suberate (Bier) as follows. Reduced yNAP-1 was dialyzed against 100 mM sodium borate, pH 8.8, for 3 h with shaking. Histones, octamer, or trypsin-treated octamer was then added, and a 0.1 volume of disuccinimidyl suberate in dimethylformamide was added to a final concentration of 2 mg/ml. Cross-linking was stopped by precipitating by adding an equal volume of 50% trichloroacetic acid. Precipitated protein was washed with acetone-HCl, then acetone, and dried. The resulting pellet was dissolved in SDS loading buffer and analyzed by 3.5% SDS-PAGE.

**Electrophoresis**—Histone-yNAP-1 complexes were resolved with 5% PAGE with 0.5 × TBE (45 mM Tris borate, 1 mM EDTA, pH 8). A variety of SDS-polyacrylamide gels of different percentage of acrylamide (30:1 acrylamide:buffer acrylamide) were used to resolve histones, yNAP-1, and cross-linked species. 15% acetic acid, urea, and Trition (ALT)-PAGE (29) was used to resolve acetylated histones. For the two-dimensional analysis of histone-yNAP-1 complexes a mixture of 20 µg of yNAP-1 and 10 µg of histone in 0.5 × TBE was electrophoresed in the first dimension into a nondenaturing 5% × 0.5% TBE polyacrylamide gel. The gel slice was then excised and electrophoresed into 15% (core histones) or 7.5% (cross-linked octamer) SDS-polyacrylamide gels.

**Far Western Blotting**—Samples were solved (25 mM Tris, 0.2 mM glycine, pH 8) electrotitransferred for 30 min at 24 V to a wetted PVDF (Immobilon, Millipore) membrane from 15% SDS- or AUT-polyacrylamide gels. In some cases the transfer followed the electrophoretic separation directly; in others the proteins in the Coomassie Blue R-stained gel were solubilized for 30 min in SDS-polyacrylamide gel buffer supplemented with SDS to 1% and then electrotransferred. The blots were washed with ethanol to remove residual stain and then subjected to a renaturing Far Western protocol described by Lieberman and Berk (30). The concentration of [35S]- or fluorescein-labeled yNAP-1 in the incubation step was about 5 µg/ml. The dried blot was then imaged with a Molecular Dynamics SF PhosphorImager or STORM combined PhosphorImager and fluorescence imager.

**RESULTS**

**Chromatin Assembly by Recombinant Yeast NAP-1**—Chicken histone octamers premixed with yNAP-1 under reducing conditions and physiological ionic strength and subsequently added to an equal mass of high molecular weight (>20 kilobase pairs) genomic chicken DNA resulted in a soluble nucleoprotein complex. Digestion of this chromatin with micrococcal nuclease for various times and subsequent deproteinization and electrophoresis yielded a ladder of 5–6 DNA frag-
ments with a repeat of 146 bp as shown in Fig. 1B. The same experiment without yNAP-1 gave only low yields of fragments up to dimers (Fig. 1A). Essentially the same yNAP-1-assisted assembly pattern was observed when linker histones were present during assembly or when the histone to DNA mass ratio varied from 0.4 to 0.8:1 (data not shown). Decreasing the input ratio of histone octamer to DNA did not change the repeat but did reduce the extent of the ladder, suggesting that the nucleosome spacing results from very closely spaced octamers along the chromatin fiber which may cooperatively interact. Close-packed dinucleosomes have been observed following micrococcal nuclease digestion of salt dialysis-produced chromatin (10).

Histone Octamer Integrity Is Essential for Uniform Nucleosome Spacing—We have observed that many core histone preparations including those obtained by acid extraction do not produce a discrete extended ladder in yNAP-1 chromatin assembly experiments (data not shown). These same histone samples can be reconstituted readily into nucleosomes with core-length DNA following a salt dialysis regimen from strongly denaturing conditions as shown in Fig. 2. The only exception was a cross-linked histone octamer prepared by DMS-cross-linking salt-stripped chromatin in 2 M NaCl. Although homogeneous by electrophoresis and sedimentation analysis (data not shown), the cross-linked octamer failed to form a core nucleosome or a yNAP-1-assembled chromatin. In an attempt to establish the requirement for the existence of a histone octamer for yNAP-1-facilitated chromatin assembly, a chicken histone octamer sample in 2 M NaCl, TE, pH 8, was brought to 6 M guanidinium HCl by the addition of solid guanidinium chloride. The guanidinium chloride was removed by dialysis of the sample against 2 M NaCl, TE, pH 8, and the renatured octamer sample was used in a yNAP-1 assembly experiment. The resulting micrococcal nuclease digestion pattern is shown in Fig. 1C and comparative densitometric traces in Fig. 1F. The renatured octamer ladder is very indistinct compared with the native octamer ladder even though the histone composition after assembly was unchanged as judged by SDS-PAGE (data not shown). We conclude from these re-
results that the formation of close-packed arrays of nucleosomes facilitated by yNAP-1 requires a high proportion of structurally intact histone octamer. This is presumably not the case with many histone preparations that at one point or another become dissociated by exposure to low ionic strengths, low pH, or denaturants. Our results suggest that for yNAP-1 to assemble a uniformly spaced chromatin the histone octamer must pre-exist or be stabilized by other factors. The inability of the DMS-cross-linked octamer to reconstitute into nucleosomes or chromatin suggests that there may be conformational changes in the octamer structure on salt dissociation from chromatin which are not consistent with the structure of the core nucleosome. The DMS-cross-linked octamer forms a complex with yNAP-1 as does the native histone octamer (see below). This together with the fact that octamers extensively DMS-cross-linked while in the nucleosome are capable of nucleosome reconstitution (31) argues against a chemical modification explanation for absence of reconstituted product.

**Nucleosome Close Packing Does Not Depend on the Underlying DNA Sequence—**Inclusion of a uniformly 32P-labeled 2,400-bp DNA fragment containing both the promoter and coding region for the PHO5 gene in the assembly mixture results in a digest pattern following electrophoresing and PhosphorImaging which is very similar to the one observed by ethidium staining (Fig. 1, D and E, without and with yNAP-1 compared with Fig. 1, A and B, respectively). There are some indications of a greater size heterogeneity in the labeled fragment particularly in the monomer and dimer fragments which might be due to sequence or end effects. Similar experiments with smaller PHO5 fragments encompassing separately the promoter and coding regions as well as with DNase I digestion (data not shown) suggest that although the former is the most likely explanation, the sequence-specific effects are minor. These results, to a first approximation, indicate that yNAP-1-facilitated chromatin assembly is not influenced by the underlying DNA sequence even on DNA that is known to contain positioned nucleosomes and a nucleosome-free gap in vivo (32) during expression of the PHO5 gene.

**Chromatin Remodeling Is a yNAP-1 Capability—**Chromatin produced by salt dialysis and subsequently digested with micrococcal nuclease contains few uniformly spaced nucleosomes as shown in Fig. 3A and reported earlier by Stein (10). Addition of yNAP-1 to the undigested chromatin and subsequent incubation at 37 °C for 3 or more hours at physiological ionic strength result in a chromatin containing uniformly spaced nucleosomes (Fig. 3B). The amount of yNAP-1 added was the same as that in a typical assembly reaction. If trypsin-treated chicken histone octamers are used no such remodeling occurs with yNAP-1 (data not shown).

**Yeast NAP-1 Forms Complexes with Chicken Core Histones and a Cross-linked Histone Octamer—**When core histones are mixed with recombinant yNAP-1 in solutions of moderate ionic strength, complexes can be resolved by nondenaturing PAGE (Fig. 4A, lanes 2–7). Two main complexes a and b are observed; c represents the position of uncomplexed yNAP-1. If a DMS-cross-linked histone octamer is complexed with yNAP-1 then only complex a is observed as well as some slower moving bands (Fig. 4B, lanes 2–7). The composition and stoichiometry of 2:1 mass ratio yNAP-1 complexes with chicken core histones as well as with DMS-cross-linked histone octamer were analyzed by two-dimensional electrophoresis (Fig. 5, A and B, respectively). The complex at position a in Figs. 4A and 5A contained yNAP-1 and all four of the core histones. Complexes with greater electrophoretic mobility than a in Fig. 5A contained progressively less histones H3 and H4 as shown by densitometry in Fig. 5C. The material at b had 50% less H3 and

![Figure 3](image)

**FIG. 3. Yeast NAP-1 remodels salt dialysis-produced chromatin.** Chicken histone octamers (20 μg) were mixed with chicken genomic DNA (20 μg, > 10 kilobase pairs) in 1 mM NaCl, 10 mM Tris, pH 8, 1 mM EDTA. Following a dialysis regimen, one half of the resulting synthetic chromatin was digested with micrococcal nuclease for various times, deproteinized, and resolved on a 1.5% agarose gel. The other half of the sample was adjusted to physiological ionic strength, and yNAP-1 was added. After a 12-h incubation at 37 °C this sample was also digested with micrococcal nuclease. Lane 1, chicken genomic DNA; lanes 2 and 10, 100-bp DNA ladder (Pharmacia); lanes 3 and 9, nucleosomal DNA from chicken erythrocyte nuclei; lanes 4–8, DNA from micrococcal nuclease-digested synthetic chromatin for 1, 5, 10, 20, and 30 min. Panel A, salt-dialysis chromatin; panel B, yNAP-1-remodeled salt dialysis chromatin.

![Figure 4](image)

**FIG. 4. Yeast NAP-1 forms complexes with chicken core histones and a cross-linked histone octamer but not with trypsin-treated histones.** Histone-yNAP complexes were resolved by native 5% 0.5 × TBE-PAGE. The anode is at the gel bottom. Panel A, chicken core histone-yNAP-1. Lanes 2–7, molar ratios of histone octamer:yNAP-1 (0, 0.06, 0.12, 0.18, 0.24, and 0.61). Lanes 8–13, molar ratios of trypsin-treated histone-yNAP-1 (0, 0.06, 0.12, 0.18, 0.24, and 0.61, respectively). Lane 1, mononucleosome sample. a represents the octamer-yNAP-1 complex, b is indeterminate complex(es), and c is the position of yNAP-1 alone or a core nucleosome. Panel B, same as panel A but with purified DMS-cross-linked chicken histone octamer. Lanes 2–7, molar ratios of histone octamer to yNAP-1 (0, 0.06, 0.12, 0.18, 0.24, 0.3). Lane 8, yNAP-1 alone. Lanes 1 and 5, mononucleosome.

H4 compared with complex a. Densitometry as well as double labeling experiments (data not shown) indicates a mass ratio of 2.5:1 NAP-1 to total histone for both the histones and cross-linked histone octamer in Fig. 5, A and B, respectively, corre-
sponding to a molar ratio of 4:1 for yNAP-1 to histone octamer. A titration of a fixed amount of yNAP-1 with increasing amounts of core histones is shown in Fig. 6. The point at which no additional band is formed lies between 3 and 4:1 yNAP-1 to histone octamer.

yNAP-1 Does Not Bind Tightly to Mono- or Polynucleosomes—If yNAP-1 can remodel salt dialysis-produced chromatin then it may be that yNAP-1 is chromatin-bound following assembly or remodeling. To examine this question 14C-labeled His$_6$ yNAP-1 was mixed with micrococcal nuclease-produced chicken chromatin fragments, and the mixture was resolved by nondenaturing PAGE as shown in Fig. 7. The chromatin fragments contained both core and linker histones (data not shown), and the bulk of the DNA was contained in trinucleosome and larger oligomers. For comparison, samples of His$_6$ yNAP-1 alone (lanes 1 and 5) and a cross-linked octamer-His$_6$ yNAP-1 complex (lane 4) were included. Most of the His$_6$ yNAP-1 migrates in the uncomplexed region whether chromatin fragments are present or not. Densitometry indicates that the yNAP-1 to DNA ratio in region a is less than 5% of that in region c of Fig. 7, lane 3. However, there is a small amount of yNAP-1 which exhibits a lower mobility in the presence of the chromatin fragments, but the band does not coincide with a chromatin fragment (panel C, lane 3). From this we conclude that yNAP-1 does not bind tightly to chromatin compared with the histone octamer and thus could act catalytically in an assembly process.

Yeast NAP-1 Binds to All Chicken Core and Linker Histones and HMG-1/HMG-2 but Not HMG-14—To determine the relative affinities of individual histones and other basic proteins to yNAP-1, affinity blotting Far Western experiments were performed by resolving the basic proteins on SDS-PAGE, electrophoretically transferring them to a PVDF membrane, renaturing the adsorbed proteins, and binding labeled yNAP-1 under condi-
 Histones Lacking Amino Termini—Trypsin-treated chicken histone octamers were prepared by trypsin digestion of salt-stripped chicken chromatin followed by hydroxyapatite chromatography. Trypsin inhibitor was added after the digestion. The sample was mixed with yNAP-1 and DNA in the same way as chicken histone octamers. SDS-PAGE indicated that the digestion had removed only the NH₂ termini of all four of the core histones (data not shown). The assembly results shown in Fig. 9, A and B, indicate almost no uniformly spaced nucleosomes exist in intact histones complexed with yNAP-1, the former in trypsin-treated histones complexed with yNAP-1. Neither band is observed with yNAP-1 or histones separately. The identity and stoichiometry of the cross-linked species are complicated by the anomalously slow migration of both histones and yNAP-1 on SDS-PAGE. Assuming that the cross-linked molecular mass is the sum of the apparent molecular masses observed for the monomeric components yNAP-1 60 kDa, core histones 14–18 kDa, then the 410-kDa species might be a tetramer or pentamer of yNAP-1 bound to a histone octamer (370 or 430 kDa). The 136-kDa cross-linked species found in both the plain and trypsin octamer samples might be a yNAP-1 dimer cross-linked to a single histone. Further experiments with cleavable cross-linkers and two-dimensional SDS-PAGE would be needed to identify unequivocally the components of the observed cross-linked species. We note that unlike nucleoplasmin (5), cross-linking of yNAP-1 alone does not produce any appreciable multimeric species, which suggests that the predominant form of yNAP-1 at micromolar concentrations is a monomer. The absence of yNAP-1 multimers in the nondenaturing PAGE experiments (see Fig. 1, lanes 2 and 8) also supports the idea that yNAP-1 does not self-associate except perhaps when bound to histones.

The Binding Affinity of yNAP-1 for Histones Is Greater Than That for More Basic Proteins and Peptides—Because many basic proteins and peptides do not migrate on SDS-PAGE due to the formation of insoluble complexes with SDS, a competition assay was devised to allow a comparison of the relative binding affinities of yNAP-1 with these proteins. The competition experiment involved complexing yNAP-1 with a candidate protein at a mass ratio of 1:1 and then incubating the complex with a PVDF membrane strip containing one-third the amount of immobilized renatured total chicken histones resolved by SDS-PAGE. The amount of yNAP-1 binding to histone H4 on the strip after equilibration overnight at 37 °C was used as a measure of the affinity of the yNAP-1 for the competitor protein. The results, shown in Table I, demonstrate that protamine, poly-L-lysine, and poly-L-arginine have an appreciably lower affinity for yNAP-1 than even histone H4. This is despite charge densities that are up to four times higher than that of histone H4. By comparison a cross-linked histone octamer has the greatest affinity for yNAP-1 compared with any of the individual core histones, which might suggest the existence of yNAP-1-yNAP-1 interactions in the octamer–yNAP-1 complex.

The Binding of yNAP-1 to Core and Linker Histones Is Pri-
The Affinity of yNAP-1 for Histone H4 Is Not Altered by Natural Acetylation—Chicken and HeLa histones acetylated chemically and by butyrate induction, respectively, were resolved by SDS-PAGE and transferred onto a PVDF membrane in 3 ml of buffer following a Far Western protocol. Fluorescein-labeled yNAP-1 bound to the core histones was detected with a fluorescence imager. Prior to probing, an equal amount of various competitors was added to the fluorescein-labeled yNAP-1 solutions. The results are expressed as percentage of fluorescein-labeled yNAP-1 binding to 200 ng of histone H4 on the membrane relative to a no-competitor sample.

### Table I

| Competitor                  | %  |
|-----------------------------|----|
| None                        | 100|
| Chicken core histones       | 17 |
| Cross-linked histone octamer| 0  |
| Protamine (Salmine)         | 70 |
| Poly-L-lysine (50 kDa)      | 45 |
| Poly-L-arginine (48 kDa)    | 45 |

**FIG. 10.** Cross-linking of yeast NAP-1 with chicken histone octamer suggests a stoichiometry of about 4:1. yNAP-1 alone and mixed with chicken histone octamer and trypsin-treated chicken histone octamer at a molar ratio of 4:1 were cross-linked with disuccinimydyl suberate at pH 9 in 0.1 M sodium borate. The cross-linked products were resolved by 3.5% SDS-PAGE. Lanes 1 and 2, electrophoretic markers; lane 2, cross-linked glyogen phosphorylase with a monomer molecular mass of 97 kDa (Sigma); lane 3, yNAP-1 alone; lane 4, yNAP-1 with trypsin-treated chicken histone octamer; lane 5, yNAP-1 with chicken histone octamer. Calculated molecular masses of the cross-linked species are indicated.

**FIG. 11.** yNAP-1 binds primarily to the amino-terminal tails of chicken core histones. Panel A, stained core histones for 0, 1, 2, 5, and 10 min of trypsin digestion after electrotransfer to a PVDF membrane, lanes 1–5, respectively. **Panel B**, Far Western blot of the membrane in panel A probed with 35S-labeled yNAP-1.

**FIG. 12.** yNAP-1 binds primarily to the nonglobal region of chicken histone H5. Panel A, stained chicken histone H5 (10 ¡g) for 0, 2, 4, 6, 8, 10, 15, 20, and 30 min of trypsin digestion followed by electrotransfer to a PVDF membrane, lanes 2–10, respectively. Lanes 1 and 11, size markers; lanes 12 and 13, histone H5 (3 ¡g of high performance liquid chromatography-purified. Panel B, Far Western blot of the membrane in the panel A probed with 35S-labeled yNAP-1. GH5 is the trypsin-resistant globular domain.

high molecular weight DNA. No additional factors or metabolites are required, only the preexistence of a histone octamer with intact NH2 termini. This result agrees with earlier work by Ishimi and co-workers (19, 20), who showed that yNAP-1 interacts in solution with core histones and places nucleosomes onto plasmid DNA as assayed by the extent of induced supercoiling. The repeat length of the yNAP-1-assembled chromatin is considerably shorter than that found in vivo. The in vitro assembly of physiologically spaced nucleosomes from core histone octamer and DNA requires the combined action of yNAP-1 and a Drosophila assembly fraction dCAF-1, a source of ATP and linker histones (15). The chromatin assembly properties of yNAP-1 in isolation suggests that components of the dCAF-1 fraction as well as linker histones may contribute to histone octamer formation and stability as well as ultimately increasing the internucleosomal separation (15). Because the histone octamer is intrinsically unstable at low concentrations and at physiological ionic strength in the absence of polymers (33), we speculate that ATP is required for dCAF-1-mediated histone octamer formation.

**DISCUSSION**

In vitro, at physiological ionic strengths, yNAP-1 facilitates the uniform placement of core histone octamers onto linear
Earlier studies have shown that yNAP-1 forms complexes with core histones (19, 20), whereas this work establishes that linker histones and HMG-1/HMG-2 bind yNAP-1 as well. The binding of yNAP-1 to these basic molecules, we show, is through histone regions, which are highly positively charged as well as structurally disordered in free solution. Both yNAP-1-facilitated chromatin assembly and remodeling require that the histone amino termini be intact and suggest that NAP-1 might function as an assembly factor rather than merely as chaperone that prevents inappropriate largely electrostatic histone-DNA interactions. The report by Kadonaga and coworkers (15) that both yeast and Drosophila NAP-1 can act in concert in vitro with a Drosophila ATP-dependent CAF-1 fraction to produce a uniformly spaced chromatin with a physiological repeat supports this notion.

The complex of yNAP-1 with a core histone octamer has a stoichiometry of 4:1, although other complexes between individual histones and NAP-1 as well as the 2A/2B dimer and 3/4 tetramer undoubtedly occur in solution depending upon the initial complexation composition and conditions. In contrast to nucleoplasmin, which exists in solution as a pentamer (5), we find NAP-1 is mainly monomeric in free solution. In this respect NAP-1 behaves more like N1/N2 which is reported to be monomeric and to bind histones H3 and H4 in vivo (36). The affinity of yNAP-1 for individual histones is roughly equal, and our chromatin assembly experiments suggest that NAP-1 is unable to promote histone octamer formation.

Although the binding of yNAP-1 to linker histones is novel, a recent report from Dimitrov and Wolffe (37) on the ability of nucleoplasmin to displace linker histones from chromatin suggests a similar interaction between nucleoplasmin and linker histones. After submission of this manuscript Rodriguez et al. (38) reported that human NAP can interact with linker histones. That yNAP-1 can bind to HMG-1/HMG-2 parallels the apparent interchangeable nature of these molecules with linker histones in chromatin (39). It is interesting that chicken HMG-14 does not bind yNAP-1 despite containing 25% basic amino acid residues (40). The recent demonstration that yeast contains linker histone (41) and HMG-1 homologs (42) provides potential physiological relevance for these in vitro observations.

The strong binding of yNAP-1 to core and linker histones is not solely an electrostatic effect, as competition experiments reveal that poly-L-lysine and poly-L-arginine do not bind yNAP-1 as tightly. Furthermore a deletion analysis of yNAP-1 demonstrates that the most negatively charged region is dispensable for nucleosome assembly and histone binding (20). Although affinity blotting shows that high levels of chemical acetylation reduce the binding of yNAP-1 to core histones, naturally acetylated histone H4 displays no differential yNAP-1 binding affinity even though the net positive charge on this segment of H4 is reduced by 50% in the tetraacetylated linker histones and HMG-1/HMG-2 bind yNAP-1 as well. The affinity of yNAP-1 for individual histones is roughly equal, and our chromatin assembly experiments suggest that NAP-1 is unable to promote histone octamer formation.

Additionally, yNAP-1 is capable of remodeling salt dialysis-produced chromatin to produce an array of close-packed nucleosomes. This property requires that the histone octamer NH$_2$ termini be intact. Recent in vitro and in vivo studies of chromatin assembly in yeast (34) demonstrate the essentiality of one or other of the NH$_2$ termini of histones H3 and H4 for chromatin assembly. Blank and Becker (1) also report that although trypsin-treated histones are assembled onto DNA, the nucleosome alignment is much less regular in Drosophila extract-assembled chromatin. NURF, a Drosophila nucleosome remodeling factor, requires the histone tails for full activity (35). Thus for assembly under physiological conditions the histone H3 and H4 NH$_2$ termini are crucial. This is in contrast to salt dialysis regimes that assemble trypsin-treated histones into nucleosomes readily. It is tempting to speculate that yNAP-1 binds to the core histone NH$_2$ termini in chromatin thereby reducing the electrostatic interaction of these tails with the DNA and thus promoting sliding or even a transient displacement of the octamer from the chromatin fiber. Our results show that the interaction of yNAP-1 with the core octamer when bound to DNA is considerably weaker than when octamer is in free solution. The weak binding of yNAP-1 to the chromatin fiber infers that it could act catalytically in the nucleus.

FIG. 13. Histone H4 acetylation does not change yNAP-1 affinity markedly. Panel A, stained histones (10 µg) electrophoresed on a 15% AUT gel and transferred to a PVDF membrane. Lane 1, chicken histones; lane 2, chemically acetylated chicken histones; lane 3, HeLa histones; lane 4, HeLa butyrate histones. Panel B, Far Western blot of the membrane in the panel A probed with 35S-labeled yNAP-1. Panel C, trace of the stained gel (panel A) and PhosphorImage (panel B) in the region of histones H2B and H4 for lane 4. Individual H4 acetylated species are indicated above the trace.
association with H2A and H2B (15, 22) suggests a transport role from the cytoplasm to the nucleus. In the nucleus NAP-1 may act in concert with the recently discovered ATP-dependent chromatin remodeling factors (46–48). The ability of NAP-1 to bind specifically to the amino termini of the core histones and the disordered regions of linker histones is intriguing as these regions are the targets for post-translational modifications as well as sites of interaction with transcriptional activator proteins (34). In this regard in vitro experiments have shown that yNAP-1 is able to facilitate the binding of a transcription factor to nucleosomal DNA (21). A possible function for NAP-1 then is to modulate access to these important histone domains. Experiments to test this hypothesis are in progress.

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