

RSC Unravels the Nucleosome

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Summary

RSC and SWI/SNF chromatin-remodeling complexes were previously reported to generate a stably altered nucleosome. We now describe the formation of hybrids between nucleosomes of different sizes, showing that the stably altered structure is a noncovalent dimer. A basis for dimer formation is suggested by an effect of RSC on the supercoiling of closed, circular arrays of nucleosomes. The effect may be explained by the interaction of RSC with DNA at the ends of the nucleosome, which could lead to the release 60–80 bp or more from the ends. DNA released in this way may be trapped in the stable dimer or lead to alternative fates such as histone octamer transfer to another DNA or sliding along the same DNA molecule.

Introduction

The coiling of DNA by histones in nucleosomes is a barrier to the entry of proteins involved in transcription, replication, and other DNA transactions (Grunstein, 1990; Kornberg and Lorch, 1999; Struhl, 1999; Wyrick et al., 1999). The barrier is high due to the tight binding of histones to DNA and consequent stability of the nucleosome. The barrier is breached at least in part through the action of chromatin-remodeling complexes. Two families of such complexes containing Swi2/Snf2 or ISWI ATPases have been described, and evidence for a third family based on the Mi-2 ATPase has been presented (Cairns, 1998; Aalfs and Kingston, 2000; Boyer et al., 2000; Vignali et al., 2000). Yeast SWI/SNF complex is implicated by microarray analysis in the transcription of a few percent of all genes and is nonessential for cell growth (Sudarsanam et al., 2000). A related complex, termed RSC, contains several homologs of SWI/SNF subunits, all of which are encoded by essential genes (Cairns et al., 1996, 1998, 1999). RSC mutants have been identified with *spt*⁻ phenotypes, pointing to a role of the RSC complex in transcription as well (Cairns et al., 1998).

Yeast and human SWI/SNF complexes and yeast RSC complex perturb the structure of nucleosomes in an ATP-dependent manner *in vitro*. Nucleosomal DNA is rendered more accessible to nuclease digestion, and DNA supercoiling of a closed circular array of nucleosomes is much diminished (Cote et al., 1994; Imbalzano et al., 1994; Cairns et al., 1996; Guyon et al., 1999; Jaskelioff et al., 2000). Despite the increase in accessibility and loss of supercoiling, the histones remain associated

with the DNA. The stability of the nucleosome is reduced, but the gross structure of the nucleosome is unaffected. This “activated” state of the nucleosome is dependent on the presence of the chromatin-remodeling complex but not on continuing ATP hydrolysis (Imbalzano et al., 1996; Cote et al., 1998; Lorch et al., 1998; Schnitzler et al., 1998).

Reduced stability of the activated nucleosome is manifested by “mobility” of the core histone octamer. The activated nucleosome formed by RSC can transfer the octamer to exogenous DNA, forming a new nucleosome (Lorch et al., 1999). SWI/SNF and also ISWI complexes promote sliding of the octamer to adjacent positions along the same DNA (Hamiche et al., 1999; Langst et al., 1999). Sliding can occur without transfer and often culminates in most stable positions of the octamer near the ends of linear DNA (Whitehouse et al., 1999).

Removal of the chromatin-remodeling complex from an activated nucleosome results in a persistently altered state (Cote et al., 1998; Lorch et al., 1998; Schnitzler et al., 1998). The alteration is revealed by an increased sedimentation rate and decreased electrophoretic mobility. The altered nucleosome retains a full complement of histones but with a diminished histone–DNA interaction, as shown by increased sensitivity to dissociation at elevated ionic strength.

An activated nucleosome formed by a chromatin-remodeling complex can, therefore, meet three fates: histone octamer transfer, sliding, or conversion to a stably altered state. Here we investigate the perturbation of the nucleosome underlying these processes. We show how the structure of the stably altered nucleosome and a previously unexplained change in DNA supercoiling of nucleosomal arrays may be related, with attendant insight into the mechanism of chromatin remodeling by RSC and SWI/SNF complexes.

Results

Formation of the Persistently Altered Nucleosome

In previous studies, a nucleosome on a small DNA fragment was converted to an activated complex by incubation with yeast RSC or human SWI/SNF in the presence of ATP, followed by the removal of the chromatin-remodeling complex by competition with excess nucleosomes or DNA (Lorch et al., 1998; Schnitzler et al., 1998). Breakdown of the activated complex yielded the original nucleosome and a smaller amount of a persistently altered form. We now find, in reactions performed with RSC and a nucleosome on a slightly longer DNA fragment (217 bp, compared with 154 bp in the original experiments with RSC), that the altered nucleosome is obtained without the addition of competing nucleosomes or DNA. The altered nucleosome is released from the complex with RSC over time (Figure 1A). Apparently, the additional DNA protruding from the ends of the 217 bp nucleosome facilitates the formation of or stabilizes the altered nucleosome. It thus accumulates despite conversion back to the original state, which is catalyzed by RSC as well.

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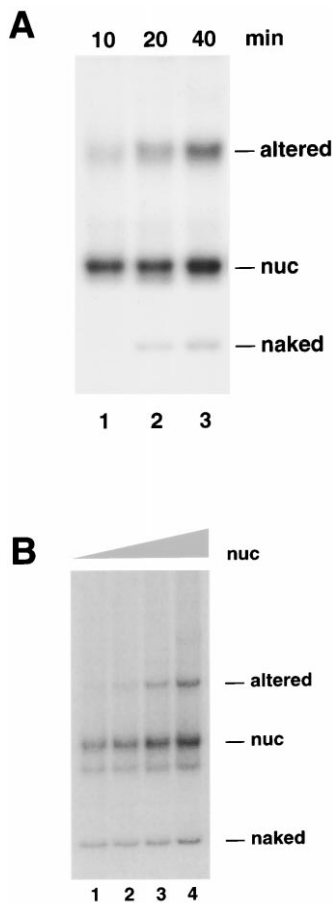


Figure 1. Formation of Stably Altered Nucleosomes

(A) Formation of altered nucleosomes without the removal of RSC. A peak maltose gradient fraction (2 μ l) of 217 bp nucleosomes (5.4 ng/ μ l) was treated with RSC (40 ng) and 0.6 mM ATP in a volume of 5 μ l at 30°C for the times indicated, followed by the addition of 0.25 μ l of 0.6 M glucose and 0.25 μ l of hexokinase (4 mg/ml), incubation for 5 min at 30°C, and polyacrylamide gel electrophoresis. Bands due to altered nucleosomes ("altered"), nucleosomes ("nuc"), and naked DNA ("naked") are indicated.

(B) Formation of altered nucleosomes depends on the nucleosome concentration. A peak maltose gradient fraction (lane 1, 0.2 μ l; lane 2, 0.4 μ l; lane 3, 0.4 μ l; and lane 4, 0.4 μ l) of 154 bp nucleosomes (4 ng/ μ l) was treated with RSC (40 ng) and 0.6 mM ATP in a volume of 5 μ l for 10 min at 30°C, followed by the addition of 1 μ g of plasmid DNA and polyacrylamide gel electrophoresis.

Formation of the altered nucleosome also proved to be strongly concentration dependent (Figure 1B). There was a more than linear increase in the amount of altered nucleosome generated with increasing levels of nucleosomes in the reaction. This behavior is consistent with an association of two nucleosomes in the altered form, as previously suggested on the basis of a greater sedimentation rate and lower electrophoretic mobility of the altered form (Lorch et al., 1998; Schnitzler et al., 1998).

The Altered Nucleosome Is a Noncovalent Dimer

The sedimentation rate and electrophoretic mobility of the altered nucleosome could be explained not only by the interaction of two nucleosomes in a dimer but also

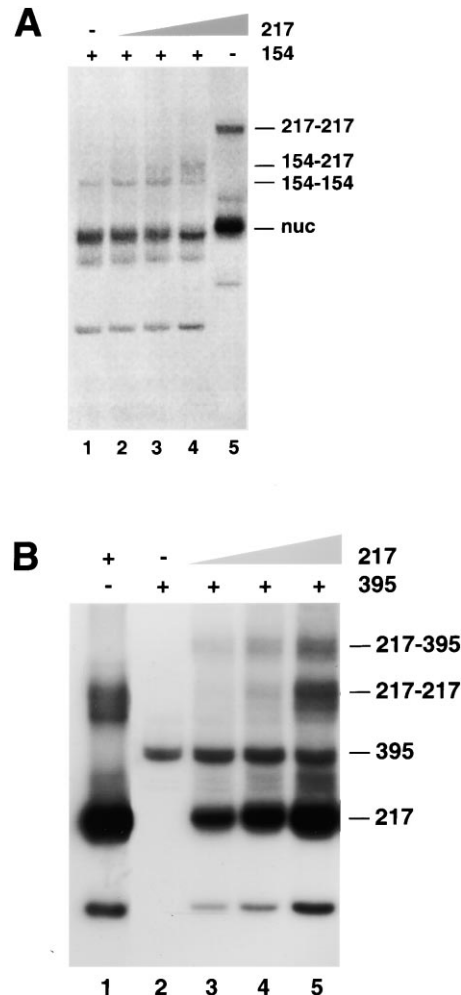


Figure 2. Formation of Hybrid Altered Nucleosomes

(A) Peak maltose gradient fractions of 217 bp nucleosomes (as in Figure 1A, lane 2, 0.7 μ l; lane 3, 1.3 μ l; lane 4, 1.7 μ l; and lane 5, 2.7 μ l) and of 154 bp nucleosomes (as in Figure 1B, 1.1 μ l) were treated with RSC (40 ng) and 0.6 mM ATP in a volume of 5 μ l for 60 min at 30°C, followed by the addition of 1 μ g of plasmid DNA and polyacrylamide gel electrophoresis. Bands due to 217 bp altered nucleosomes (217-217), 154 bp altered nucleosomes (154-154), and hybrid (154-217) altered nucleosomes are indicated.

(B) Peak maltose gradient fractions of 217 bp nucleosomes (36.6 ng/ μ l; lane 1, 1 μ l; lane 3, 0.25 μ l; lane 4, 0.5 μ l; and lane 5, 1 μ l) and of 395 bp nucleosomes (23 ng/ μ l; lanes 2-5, 3.5 μ l) were treated with RSC (40 ng) and 0.6 mM ATP in a volume of 5 μ l for 15 min at 30°C, followed by the addition of 1 μ g of plasmid DNA and polyacrylamide gel electrophoresis. Bands due to hybrid (217-395) altered nucleosomes, 217 bp altered nucleosomes (217-217), 395 bp nucleosomes (395), and 217 bp nucleosomes (217) are indicated.

by a conformational change of the nucleosome or by the association of additional proteins, such as RSC subunits. Strong evidence for a dimer was obtained by the formation of hybrids between nucleosomes containing DNAs of different lengths. Although nucleosomes containing 154 and 217 bp DNAs are nearly indistinguishable in gels, altered forms of these nucleosomes exhibit quite different electrophoretic mobilities (Figure 2A, lanes 1 and 5). Addition of increasing amounts of the 217 bp to the 154 bp nucleosome resulted in the gradual

appearance of a new species of intermediate mobility, which we attribute to a 154–217 bp altered nucleosome hybrid (Figure 2A, lanes 2–4).

A similar experiment was performed with nucleosomes containing 217 bp and 395 bp DNAs. In this case, the starting nucleosomes were well resolved (Figure 2B, lanes 1 and 2). Addition of increasing amounts of the 217 bp to the 395 bp nucleosome resulted in the gradual appearance of a new species of mobility greater than that of the 217 bp altered nucleosome, which we attribute to a 217–395 bp altered nucleosome hybrid (Figure 2B, lanes 3–5).

RSC Catalyzes Both Histone Octamer Transfer and Sliding

It may be asked whether a hybrid can be formed between a nucleosome and a naked DNA fragment. To this end, a mixture of the 217 bp nucleosome and naked 395 bp DNA was treated with RSC and ATP followed by the removal of RSC. A new band appeared in the gel, corresponding to the fastest migrating of a series of bands formed by a 395 bp nucleosome (Figure 3A). Evidently, histone octamer transfer occurred from the 217 bp nucleosome to the 395 bp naked DNA.

The observation of multiple bands from a 395 bp nucleosome was anticipated from previous studies showing such patterns for nucleosomes on long DNA fragments (O'Donohue et al., 1994; Studitsky et al., 1995; Hamiche et al., 1999; Langst et al., 1999). The bands arise from multiple positions of the nucleosome on the DNA. In our case, the positions could be mapped with reference to restriction enzyme cleavage sites in the middle of the fragment (EcoRI sites 154 bp from one end and 216 bp from the other) and nearer the two ends of the fragment (DraI site 96 bp from one end and PvuII site 123 bp from the other end). The results indicated that the slowest migrating nucleosome was positioned near the middle of the fragment and the fastest migrating nucleosomes were near the ends (data not shown). Nucleosomes formed on the 395 bp DNA fragment by histone octamer transfer were, therefore, positioned near the ends.

Histone octamer transfer might have occurred directly to the ends of the 395 bp fragment, or transfer might have been initially to the middle of the fragment followed by sliding to the ends. Other chromatin-remodeling complexes, including SWI/SNF, NURF, and CHRAC, have been shown to catalyze nucleosome sliding, often from the middle to the ends of DNA fragments. To test for the capacity of RSC to catalyze sliding, 395 bp DNA with nucleosomes at multiple positions was fractionated by density gradient centrifugation, and a fraction with nucleosomes in the middle was treated with RSC and ATP. The results showed rapid sliding to the ends (Figure 3B).

RSC Promotes Dissociation of DNA from the Ends of the Nucleosome

A propensity for sliding of nucleosomes to the ends of DNA fragments could reflect a property of either the remodeling complexes or the nucleosome. The remodeling complexes might be unable to function near DNA ends, or nucleosomes might be inherently more stable

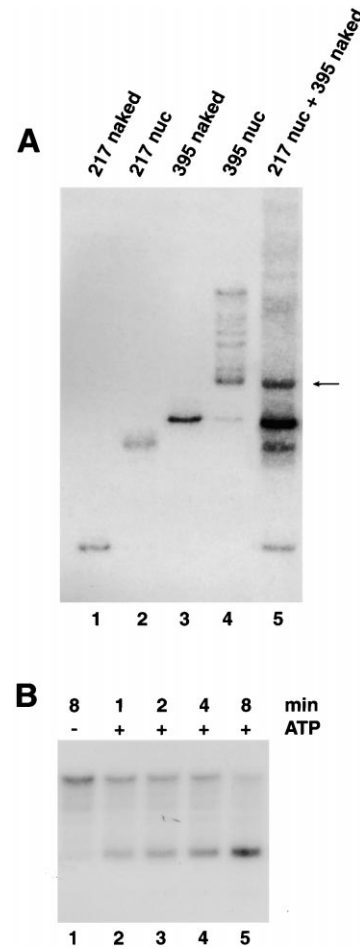


Figure 3. RSC Catalyzes Both Histone Octamer Transfer and Nucleosome Sliding

(A) Histone octamer transfer. A peak maltose gradient fraction of 217 bp nucleosomes (9.7 ng/ μ l; lane 2, 0.4 μ l and lane 5, 3.3 μ l) and 395 bp DNA (lane 3, 3 ng and lane 5, 27 ng) were treated with RSC (200 ng) and 0.6 mM ATP in a volume of 5 μ l for 60 min at 30°C, followed by the addition of 1.5 μ g of plasmid DNA and polyacrylamide gel electrophoresis. Markers of 217 bp DNA (lane 1, 4 ng) and 395 bp nucleosomes (lane 4, 6 ng) were run in the gel as well. The band due to 395 bp nucleosomes formed by histone octamer transfer is indicated by an arrow.

(B) Nucleosome sliding. A maltose gradient fraction enriched for nucleosomes at a central location on 395 bp DNA (1.9 ng/ μ l, 1.6 μ l) was treated with RSC (40 ng) and 0.6 mM ATP (+) or not (–) in a volume of 5 μ l for the times indicated at 30°C, followed by the addition of 1 μ g of plasmid DNA and polyacrylamide gel electrophoresis.

at DNA ends. To investigate whether RSC can function at DNA ends, we monitored the joining of 154 bp nucleosomes by DNA ligase. The ends of the DNA in this nucleosome are virtually inaccessible for ligation (Figure 4, lane 1). In the presence of RSC and ATP, the inhibition was relieved (Figure 4, lane 2). By contrast, RSC and ATP had no effect on the ligation of naked 154 bp DNA (Figure 4, lanes 3 and 4). RSC and ATP also did not influence the ligation of the 217 bp nucleosome (data not shown), presumably because the additional DNA protruding from the ends of this larger particle was fully

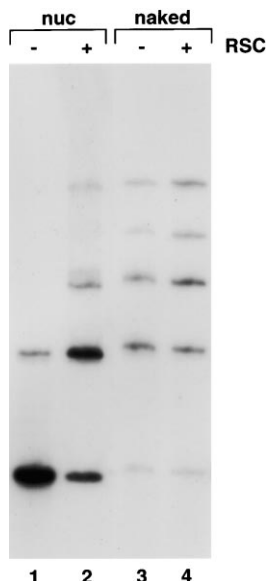


Figure 4. RSC Releases DNA from the Ends of the Nucleosome
A peak maltose gradient fraction of 154 bp nucleosomes (10.5 ng/ μ l, 3.5 μ l, lanes 1 and 2) and 154 bp DNA (11 ng, lanes 3 and 4) were treated with RSC (200 ng) as indicated (+), 0.6 mM ATP, and T4 DNA ligase (250 U, NEB) in a volume of 5 μ l for 60 min at 30°C, followed by extraction of the DNA and polyacrylamide gel electrophoresis.

accessible to ligase and of sufficient length to bridge between nucleosomes in a covalent dimer.

RSC Alters the Topology of DNA Coiling in the Nucleosome

Previous experiments with SWI/SNF complex revealed an effect on the topology of DNA coiling in the nucleosome, but no interpretation was put forward (Guyon et al., 1999; Jaskelioff et al., 2000). Here we present similar results with RSC, and we describe controls that justify interpretation in terms of interaction with DNA near the points of entry and exit from the nucleosome (“ends” of the nucleosome).

A supercoiled plasmid of about 3 kb was complexed with histones to form a circular array of nucleosomes. Following treatment with RSC, ATP, and *E. coli* topoisomerase I, the DNA was extracted and analyzed in agarose gels containing 1.3 μ M chloroquine, which adds about five positive supercoils and so brings highly negatively supercoiled molecules within the range of resolution in the gel. The starting plasmid, relaxed to near zero supercoiling by topoisomerase in the absence of any histone constraint and then positively supercoiled by chloroquine, formed the expected distribution of bands in the gel (Figure 5A, lane 2). Positive supercoiling was confirmed by analysis in a two-dimensional (2D) gel containing no chloroquine in the first dimension and 1.3 μ M chloroquine in the second (Figure 5C, left panel). Plasmids bearing nucleosomes gave broader distributions, shifted by an average Δ Lk of -10 or more (Figure 5B; negative supercoiling confirmed by 2D analysis in Figure 5C, middle panel). Since the Δ Lk associated with nucleosome formation is about -1 , the circular arrays contained, on average, ten or more nucleosomes. Fi-

nally, upon treatment with RSC and ATP, the broad distributions were shifted back to roughly half the negative Δ Lk value before treatment (Figure 5B; negative supercoiling confirmed by 2D analysis in Figure 5C, right panel).

In order to relate the effect of RSC and ATP to a change in topology of the nucleosome, two controls are required. First, the contribution to the supercoiling from RSC binding directly to the plasmid DNA must be considered. The magnitude of this effect was determined by the addition of RSC to naked plasmid DNA and treatment with topoisomerase I (Figure 5A). Slight positive supercoiling was observed at a level of RSC ten times greater than that used in the experiments with nucleosomal arrays. The effect of RSC directly on the plasmid DNA could, therefore, be neglected.

The second control concerns the possible loss of nucleosomes due to the action of RSC. This point was addressed by determination of restriction endonuclease site accessibility before and after the action of RSC. Circular arrays containing more than ten nucleosomes (Figure 6, lanes 4, 5, 8, and 9; histone to DNA ratio comparable to that in Figure 5B, lanes 3 and 4) or arrays containing 40% fewer nucleosomes (Figure 6, lanes 2, 3, 6, and 7; histone to DNA ratio comparable to that in Figure 5B, lanes 1 and 2) were treated with RSC and ATP followed by the removal of ATP and digestion with restriction endonucleases to release a DNA fragment. Arrays containing more than ten nucleosomes showed restriction site protection, yielding less fragment than naked DNA (Figure 6, compare lane 4 with lane 1), and there was no change in protection upon treatment with RSC and ATP (Figure 6, compare lane 5 with lane 4 and lane 9 with lane 8). Nucleosome loss to the extent required to explain a nearly 50% decrease in supercoiling would have been readily detectable, since the yield of restriction fragment was much greater from arrays containing 40% fewer nucleosomes (Figure 6, compare lanes 4 and 5 with lanes 2 and 3, and compare lanes 8 and 9 with lanes 6 and 7).

The supercoiling change of circular nucleosomal arrays induced by RSC and ATP was partially maintained following the removal of ATP (Figure 7, note the point of highest intensity in the band pattern shifts upon treatment with RSC and ATP for all levels of histones used, but the magnitude of the shift is less than in Figure 5B, where ATP was constantly present). The change that persisted following the removal of ATP could reflect the formation of stably altered nucleosomes in the circular arrays. The supercoiling change may have been less than that in the presence of ATP because formation of the stably altered state is maximally 25%–50% efficient (Lorch et al., 1998; Schnitzler et al., 1998).

Others have reported a loss of the supercoiling change induced by SWI/SNF complex following the removal of ATP (Jaskelioff et al., 2000). In view of the extensive homology between RSC and SWI/SNF and of the close similarity of their mechanisms (Boyer et al., 2000), the discrepancy with our results most likely reflects an experimental difference. For example, a persistent supercoiling change may not have been evident at the level of histones used in the past, since the supercoiling change decreases following the removal of ATP and further decreases as the level of histones is diminished (Figure 7).

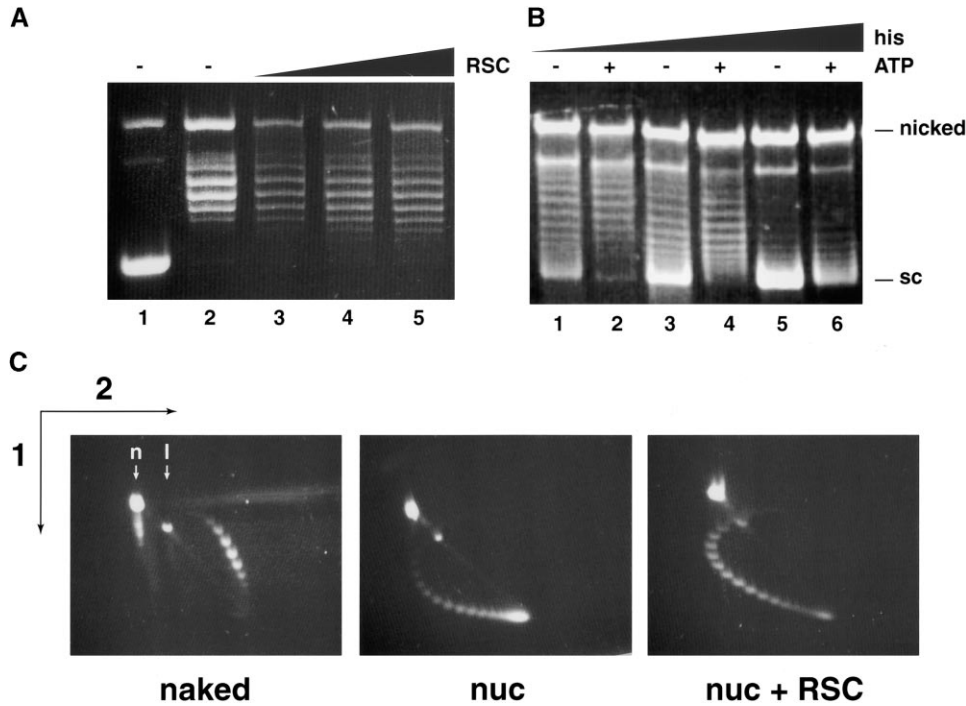


Figure 5. Supercoiling Change Induced by RSC and ATP

(A) Binding of RSC causes slight positive supercoiling of closed circular DNA. Supercoiled plasmid DNA (0.1 μ g) was treated with RSC (lane 3, 1.2 μ g; lane 4, 2.4 μ g; and lane 5, 3.6 μ g) and 0.6 mM ATP in a volume of 15 μ l for 20 min at 30°C followed by incubation with topoisomerase I (Promega; none in lane 1, 1 U in lanes 2-5) for 60 min at 37°C, extraction, and agarose gel electrophoresis. Bands due to nicked circular ("nicked") and closed supercoiled circular ("sc") DNAs are indicated at the far right.

(B) RSC and ATP cause a decrease in negative supercoiling of nucleosomal arrays. Complexes of supercoiled DNA (0.15 μ g) with histone octamers (lanes 1 and 2, 0.15 μ g; lanes 3 and 4, 0.3 μ g; and lanes 5 and 6, 0.4 μ g) were treated with RSC (0.4 μ g) and 0.6 mM ATP (+) or not (-) in a volume of 15 μ l for 20 min at 30°C followed by incubation with topoisomerase I (1 U) for 60 min at 37°C, extraction, and agarose gel electrophoresis.

(C) DNA from reactions identical with those in (A) and (B) were analyzed in 2D gels as follows: lane 2 of (A) in the left panel ("naked"), lane 3 of (B) in the middle panel ("nuc"), and lane 4 of (B) in the right panel. First and second directions of electrophoresis are indicated. Nicked ("n") and linear ("l") DNAs, migrating identically in both dimensions, provided reference markers for the supercoiled DNAs.

Discussion

The consequences of chromatin remodeling by RSC and SWI/SNF complexes are paradoxical; nuclease digestion sites are exposed and supercoiling diminishes, nor-

mally indicative of nucleosome loss, yet histones remain in place on the DNA. The formation of a stably altered form of the nucleosome by remodeling complexes is also surprising, inasmuch as the histone octamer, determinant of nucleosome structure, can be fixed by cross-linking without interfering with remodeling (Bazett-Jones et al., 1999). A simple explanation for all these findings is that RSC and SWI/SNF complexes release DNA from the ends of the nucleosome. The following line of argument from the supercoiling change induced by these complexes suggests that the release of DNA may be extensive.

A large, positive supercoiling change can be interpreted in three ways (see, for example, Prunell, 1998, and De Lucia et al., 1999). RSC may release 60–80 bp of DNA or more from the histone octamer surface, starting from the ends and invading the inner turn of DNA around the nucleosome; RSC may interact with the DNA entering and exiting the nucleosome, creating a positive node a significant fraction of the time; or RSC may impart a positive twist to the DNA. The available evidence points to the first interpretation. The extensive exposure of nucleosomal DNA to nuclease attack, the enhanced accessibility of the ends of the DNA (revealed by ligation), the persistence of part of the supercoiling change (trapped by stable dimer formation) in the absence of

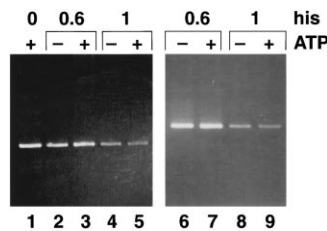


Figure 6. No Loss of Nucleosomes upon Treatment of Circular Nucleosomal Arrays with RSC and ATP

Complexes of supercoiled DNA (0.15 μ g) with histone octamers (labeled "his"; lane 1, no histone octamers; lanes 2, 3, 6, and 7, 0.18 μ g; lanes 4, 5, 8, and 9, 0.3 μ g) were treated with RSC (0.4 μ g) and 0.6 mM ATP (+) or not (-) in a volume of 15 μ l for 20 min at 30°C, followed by incubation with hexokinase (100 μ g) and glucose (0.6 μ mole) for 20 min at 30°C for the removal of ATP and then digestion with NdeI (10 U) and Aval (10 U) (lanes 1-5) or with PvuII (10 U) (lanes 6-9) for 1 hr at 37°C. Finally, DNA was extracted and analyzed by electrophoresis in a 7% polyacrylamide gel containing Tris-borate-EDTA buffer.

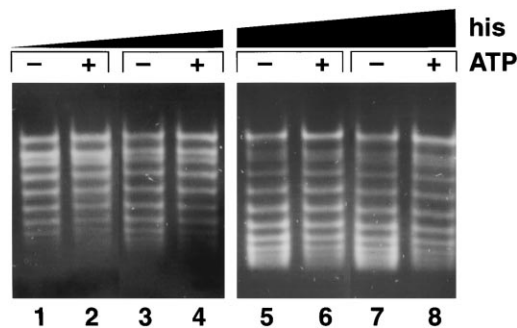


Figure 7. Supercoiling Change Induced by RSC and ATP Is Partially Maintained Following the Removal of ATP

Complexes of supercoiled DNA (0.15 μg) with histone octamers (labeled "his"; lanes 1 and 2, 0.12 μg ; lanes 3 and 4, 0.15 μg ; lanes 5 and 6, 0.18 μg ; and lanes 7 and 8, 0.21 μg) in a volume of 15 μl containing 0.6 mM ATP were treated with hexokinase (100 μg) and glucose (0.6 μmole) for 20 min at 30°C for the removal of ATP before (lanes 1, 3, 5, and 7, labeled -ATP) or after (lanes 2, 4, 6, and 8, labeled +ATP) treatment with RSC (0.4 μg) for 20 min at 30°C. Topoisomerase I (1 U) was added followed by incubation for 60 min at 37°C. Finally, DNA was extracted and analyzed by agarose gel electrophoresis.

ATP, the very formation of a stable dimer (reflecting interaction of DNA from one nucleosome with the histone surface of the other), and the catalysis of histone octamer transfer and sliding are consistent with unraveling 60–80 bp or more and are explained by the other interpretations less well or not at all. While the structural consequences of unraveling so much DNA are unknown, interactions of histone fold regions of H2A and H2B with DNA in the nucleosome are confined to about 40 bp at each end (Luger et al., 1997), so the unraveled particle might resemble a complex of an H3-H4 tetramer with DNA. The average ΔLk associated with formation of such a tetramer-DNA complex is -0.5 (Sivolob et al., 2000), similar to the residual supercoiling of the nucleosome following treatment with RSC or SWI/SNF complex. Disruption of H2A and H2B histone fold-DNA contacts would also be consistent with the results of a cross-linking study showing perturbation of H2A tail-DNA contacts by SWI/SNF complex (Lee et al., 1999).

A further argument for unraveling of DNA from the ends of the nucleosome by chromatin-remodeling complexes comes from the work of Widom and colleagues on the spontaneous (uncatalyzed) process. Unraveling was originally proposed as a basis for invasion of the nucleosome by an exonuclease (Prunell and Kornberg, 1978). It was directly demonstrated by the kinetics of restriction endonuclease digestion at sites within the nucleosome and was suggested to play a role in gene regulation by exposing internal sequences for interaction with DNA binding regulatory proteins (Anderson and Widom, 2000).

Experimental Procedures

Nucleosomes

The formation of nucleosomes on a 154 bp DNA fragment with the use of rat liver histones and stepwise dilution from 2 M NaCl followed by maltose gradient sedimentation was as described (Lorch et al., 1999). The procedure was the same for a 217 bp fragment prepared

by cleavage of pUC19 with NdeI and EcoRI and for a 395 bp fragment cleaved with NdeI and Aval from a pUC19 derivative containing the nucleosome-positioning sequence of the *Xenopus laevis* 5S rRNA gene as described (Lorch et al., 1999), and a consensus Gal4 binding 17-mer inserted at the EcoRI site. Sedimentation of the 395 bp nucleosomes in a maltose gradient, as described, yielded fractions enriched for nucleosomes in the middle or at the ends of the fragment.

Arrays of nucleosomes were formed on the pUC19 derivative described above, isolated in supercoiled form from *E. coli*. The supercoiled DNA was purified by agarose gel electrophoresis. A mixture of the purified DNA (1 μg) and rat liver histone octamers in the amount indicated in 5 μl of 1.2 M NaCl was diluted by the addition of 0.1 mg/ml BSA, 10 mM Tris-Cl (pH 7.5) to 0.8 M NaCl and then to 0.5 M NaCl with incubation for 10 min at 37°C at each stage, followed by a final dilution to 50 mM NaCl, 20 mM HEPES (pH 7.5), 3 mM MgCl_2 .

Chromatin Remodeling

All reactions with RSC and ATP contained 15 mM HEPES (pH 7.5), 3 mM MgCl_2 , 10 mM potassium acetate, and 75 $\mu\text{g/ml}$ BSA. Reaction mixtures were analyzed directly by electrophoresis in 3% polyacrylamide gels containing 10 mM Tris-Cl (pH 7.5) and 1 mM EDTA, or, alternatively, the DNA was extracted and analyzed in 0.9% agarose (Seakem agarose GTG, BMA) gels in TAE buffer (40 mM Tris-acetate, 1 mM EDTA [pH 8.0]) containing 1.3 μM chloroquine. For 2D agarose gel analysis, the gel was soaked after first dimension electrophoresis in TAE buffer containing 2.6 mM chloroquine for 6–10 hr and then run in the second dimension in TAE buffer containing 2.6 mM chloroquine. Gels containing ^{32}P -labeled DNA fragments were autoradiographed or quantitated on a PhosphorImager. Gels containing supercoiled DNA were stained with ethidium bromide and photographed.

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References

- Aalfs, J.D., and Kingston, R.E. (2000). What does "chromatin remodeling" mean? *Trends Biochem. Sci.* 25, 548–555.
- Anderson, J.D., and Widom, J. (2000). Sequence and position-dependence of the equilibrium accessibility of nucleosomal DNA target sites. *J. Mol. Biol.* 296, 979–987.
- Bazett-Jones, D.P., Cote, J., Landel, C.C., Peterson, C.L., and Workman, J.L. (1999). The SWI/SNF complex creates loop domains in DNA and polynucleosome arrays and can disrupt DNA-histone contacts within these domains. *Mol. Cell Biol.* 19, 1470–1478.
- Boyer, L.A., Logie, C., Bonte, E., Becker, P.B., Wade, P.A., Wolffe, A.P., Wu, C., Imbalzano, A.N., and Peterson, C.L. (2000). Functional delineation of three groups of the ATP-dependent class of chromatin remodeling enzymes. *J. Biol. Chem.* 275, 18864–18870.
- Cairns, B.R. (1998). Chromatin remodeling machines: Similar motors, ulterior motives. *Trends Biochem. Sci.* 23, 20–29.
- Cairns, B.R., Lorch, Y., Li, Y., Lacomis, L., Erdjument-Bromage, H., Tempst, P., Laurent, B., and Kornberg, R.D. (1996). RSC, an abundant and essential chromatin remodeling complex. *Cell* 87, 1249–1260.
- Cairns, B.R., Erdjument-Bromage, H., Tempst, P.F.W., and Kornberg, R.D. (1998). Two actin-related proteins are shared functional components of the chromatin-remodeling complexes RSC and SWI/SNF. *Mol. Cell* 2, 639–651.
- Cairns, B.R., Schlichter, A., Erdjument-Bromage, H., Tempst, P., Kornberg, R.D., and Winston, F. (1999). Two functionally distinct forms of the RSC nucleosome-remodeling complex, containing essential AT hook, BAH, and bromodomains. *Mol Cell* 4, 715–723.
- Cote, J., Quinn, J., Workman, J.L., and Peterson, C.L. (1994). Stimu-

- lation of GAL4 derivative binding to nucleosomal DNA by the yeast SWI/SNF complex. *Science* 265, 53–60.
- Cote, J., Peterson, C.L., and Workman, J.L. (1998). Perturbation of nucleosome core structure by the SWI/SNF complex persists after its detachment, enhancing subsequent transcription factor binding. *Proc Natl Acad Sci U S A* 95, 4947–4952.
- De Lucia, F., Alilat, M., Sivolob, A., and Prunell, A. (1999). Nucleosome dynamics. III. Histone tail-dependent fluctuation of nucleosomes between open and closed DNA conformations. Implications for chromatin dynamics and the linking number paradox. A relaxation study of mononucleosomes on DNA minicircles. *J. Mol. Biol.* 285, 1101–1119.
- Grunstein, M. (1990). Histone function in transcription. *Annu. Rev. Cell Biol.* 6, 643–678.
- Guyon, J.R., Narlikar, G.J., Sif, S., and Kingston, R.E. (1999). Stable remodeling of tailless nucleosomes by the human SWI-SNF complex. *Mol. Cell. Biol.* 19, 2088–2097.
- Hamiche, A., Sandaltzopoulos, R., Gdula, D.A., and Wu, C. (1999). ATP-dependent histone octamer sliding mediated by the chromatin remodeling complex NURF. *Cell* 97, 833–842.
- Imbalzano, A.N., Kwon, H., Green, M.R., and Kingston, R.E. (1994). Facilitated binding of TATA-binding protein to nucleosomal DNA. *Nature* 370, 481–485.
- Imbalzano, A.N., Schnitzler, G.R., and Kingston, R.E. (1996). Nucleosome disruption by human SWI/SNF is maintained in the absence of continued ATP hydrolysis. *J. Biol. Chem.* 271, 20726–20733.
- Jaskelioff, M., Gavin, I.M., Peterson, C.L., and Logie, C. (2000). SWI-SNF-mediated nucleosome remodeling: role of histone octamer mobility in the persistence of the remodeled state. *Mol. Cell. Biol.* 20, 3058–3068.
- Kornberg, R.D., and Lorch, Y. (1999). Twenty-five years of the nucleosome, fundamental particle of the eukaryote chromosome. *Cell* 98, 285–294.
- Langst, G., Bonte, E.J., Corona, D., and Becker, P.B. (1999). Nucleosome movement by CHRAC and ISWI without disruption or transdisplacement of the histone octamer. *Cell* 97, 843–852.
- Lee, K.M., Kingston, R.E., and Hayes, J.J. (1999). hSWI/SNF complex disrupts interactions between the H2A N-terminal tail and nucleosomal DNA. *Biochemistry* 38, 8423–8429.
- Lorch, Y., Cairns, B., Zhang, M., and Kornberg, R. (1998). Activated RSC-nucleosome complex and persistently altered form of the nucleosome. *Cell* 94, 29–34.
- Lorch, Y., Zhang, M., and Kornberg, R.D. (1999). Histone octamer transfer by a chromatin-remodeling complex. *Cell* 96, 389–392.
- Luger, K., Mader, A.W., Richmond, R., Sargent, D.F., and Richmond, T.J. (1997). Crystal structure of the nucleosome core particle at 2.8 Å resolution. *Nature* 389, 251–260.
- O'Donohue, M.-F., Duband-Goulet, I., Hamiche, A., and Prunell, A. (1994). Octamer displacement and redistribution in transcription of single nucleosomes. *Nucleic Acids Res.* 22, 937–945.
- Prunell, A. (1998). A topological approach to nucleosome structure and dynamics: the linking number paradox and other issues. *Biophys. J.* 74, 2531–2544.
- Prunell, A., and Kornberg, R.D. (1978). Relation of nucleosomes to DNA sequences. *Cold Spring Harbor Symp. Quant. Biol.* 42, 103–108.
- Schnitzler, G., Sif, S., and Kingston, R. (1998). Human SWI/SNF interconverts a nucleosome between its base state and a stable remodeled state. *Cell* 94, 17–27.
- Sivolob, A., De Lucia, F., Alilat, M., and Prunell, A. (2000). Nucleosome dynamics. VI. Histone tail regulation of tetrasome chiral transition. A relaxation study of tetrasomes on DNA minicircles. *J. Mol. Biol.* 295, 55–69.
- Struhl, K. (1999). Fundamentally different logic of gene regulation in eukaryotes and prokaryotes. *Cell* 98, 1–4.
- Studitsky, V.M., Clark, D.J., and Felsenfeld, G. (1995). Overcoming a nucleosomal barrier to transcription. *Cell* 83, 19–27.
- Sudarsanam, P., Iyer, V.R., Brown, P.O., and Winston, F. (2000). Whole-genome expression analysis of snf/swi mutants of *Saccharomyces cerevisiae*. *Proc. Natl. Acad. Sci. USA* 97, 3364–3369.
- Vignali, M., Hassan, A.H., Neely, K.E., and Workman, J.L. (2000). ATP-dependent chromatin-remodeling complexes. *Mol. Cell. Biol.* 20, 1899–1910.
- Whitehouse, I., Flaus, A., Cairns, B.R., White, M.F., Workman, J.L., and Owen-Hughes, T. (1999). Nucleosome mobilization catalysed by the yeast SWI/SNF complex. *Nature* 400, 784–787.
- Wyrick, J.J., Holstege, F.C., Jennings, E.G., Causton, H.C., Shore, D., Grunstein, M., Lander, E.S., and Young, R.A. (1999). Chromosomal landscape of nucleosome-dependent gene expression and silencing in yeast. *Nature* 402, 418–421.