

The Interactions of Yeast SWI/SNF and RSC with the Nucleosome before and after Chromatin Remodeling*

Received for publication, November 20, 2000, and in revised form, January 23, 2001
Published, JBC Papers in Press, January 24, 2001, DOI 10.1074/jbc.M10470200

Sarojini M. Sengupta‡, Michael VanKanegan‡, Jim Persinger‡, Colin Logie§¶, Bradley R. Cairns||, Craig L. Peterson§, and Blaine Bartholomew‡**

From the ‡Program in Molecular Biology, Microbiology, and Molecular Biology and Department of Biochemistry and Molecular Biology, Southern Illinois University School of Medicine, Carbondale, Illinois 62901-4413, the §Program in Molecular Medicine and Department of Biochemistry and Molecular Biology, University of Massachusetts Medical School Worcester, Massachusetts 01605, and the ||Huntsman Cancer Institute and Department of Oncological Sciences, University of Utah School of Medicine, Salt Lake City, Utah 84112

Interactions of the yeast chromatin-remodeling complexes SWI/SNF and RSC with nucleosomes were probed using site-specific DNA photoaffinity labeling. 5 S rDNA was engineered with photoreactive nucleotides incorporated at different sites in DNA to scan for the subunits of SWI/SNF in close proximity to DNA when SWI/SNF is bound to the 5 S nucleosome or to the free 5 S rDNA. The Swi2/Snf2 and Snf6 subunits of SWI/SNF were efficiently cross-linked at several positions in the nucleosome, whereas only Snf6 was efficiently cross-linked when SWI/SNF was bound to free DNA. DNA photoaffinity labeling of RSC showed that the Rsc4 subunit is in close proximity to nucleosomal DNA and not when RSC is bound to free DNA. After remodeling, the Swi2/Snf2 and Rsc4 subunits are no longer detected near the nucleosomal DNA and are evidently displaced from the surface of the nucleosome, indicating significant changes in SWI/SNF and RSC contacts with DNA after remodeling.

An important element of eukaryotic gene regulation is the dynamic nature of chromatin because of the ability of various protein complexes to alter the structure of chromatin. Chromatin is altered by chemical modification of the core histone proteins through acetylation, phosphorylation, methylation, and ubiquitination. Acetylation of the N-terminal tails has been shown to play a key role in the regulation of gene expression (1–4). Another approach to alter chromatin structure uses the mechanical energy provided by hydrolysis of the γ -phosphate of ATP without covalent modification of the nucleosome. ATP-dependent chromatin-remodeling complexes have been purified from yeast, fly, frog, and human and can range in subunit complexity from 2 to 15 different subunits/complex (5–13). These chromatin-remodeling machines have been shown *in vitro* to 1) enhance accessibility of nucleosomal DNA without the loss of the nucleosome, 2) alter the path of DNA in the nucleosome, 3) cause sliding of the nucleosome along DNA, and 4) promote octamer transfer to another DNA (14, 15). As of now, little is known about how these enzymes interact with the

nucleosome and how their contacts with the nucleosome change upon the binding and hydrolysis of ATP.

The first of these complexes to be extensively purified and characterized was the SWI/SNF complex from *Saccharomyces cerevisiae* (13, 16). The yeast SWI/SNF complex has 11 different subunits and a total molecular mass of \sim 2 MDa. The largest subunit of this complex, Swi2/Snf2, is a 194-kDa protein containing seven highly conserved subdomains corresponding to the DNA-dependent ATPase domain and a bromodomain located toward the C terminus. Single amino acid changes in the DNA-dependent ATPase domain eliminate both the ATPase activity of Swi2/Snf2 and the chromatin-remodeling activity of SWI/SNF (13, 17). The next largest subunit of SWI/SNF, Swi1 (148 kDa), had been shown to interact with DNA by UV cross-linking of SWI/SNF bound to naked DNA and contains an AT-rich interactive domain DNA-binding domain that may be involved in SWI/SNF binding of DNA (18). The RSC complex is another ATP-dependent chromatin-remodeling complex found in yeast that is closely related to SWI/SNF. The Sth1 subunit of RSC is 72% identical over 661 amino acid residues of Swi2/Snf2 (11). Three subunits of SWI/SNF, Swi3, Snf5, and Swp73, are also paralogues of RSC components; similar subunits are also found in the human and *Drosophila* SWI/SNF complexes (14). Two subunits of SWI/SNF are shared with the RSC complex and are the actin-related proteins Arp7 and Arp9 (19, 20). The ability of Arp7 and Arp9 to bind or hydrolyze ATP appears not to be important for their role in chromatin remodeling (20).

Direct correlation of the ATPase activity of the Swi2/Snf2 subunit with the chromatin-remodeling activity of SWI/SNF through mutational analysis indicates that the Swi2/Snf2 subunit is the catalytic core of SWI/SNF. These observations are also reflected in the mutational analysis of Swi2/Snf2 homologs in other SWI/SNF-like complexes. Purified recombinant Swi2-like proteins, human BRG1 and hBRM, and the *Drosophila* ISWI proteins possess some or essentially complete chromatin-remodeling activity characteristic of their respective intact multi-subunit complexes (9, 21–23). Although the mechanical energy for chromatin remodeling is provided through the ATP hydrolysis of Swi2/Snf2 or its homologs, there is no data showing the direct interaction of the Swi2/Snf2 subunit with the nucleosome or DNA. The ATPase activity of SWI/SNF and RSC is equally stimulated by naked DNA or nucleosomes. UV cross-linking of SWI/SNF bound to naked DNA did not show any Swi2/Snf2 cross-linking but instead cross-linked a protein with an electrophoretic mobility corresponding to the Swi1 subunit with

* This work was supported by the Public Health Service Grant GM48413 from National Institutes of Health (NIGMS). The costs of publication of this article were defrayed in part by the payment of page charges. This article must therefore be hereby marked "advertisement" in accordance with 18 U.S.C. Section 1734 solely to indicate this fact.

¶ Present address: Nijmegen University, Moleculaire Biologie, Toernooiveld 1, 6525-EDNijmegen, The Netherlands.

** To whom correspondence should be addressed. Tel.: 618-453-6437; Fax: 618-453-6440; E-mail: bbartholomew@siu.edu.

lesser amounts of two proteins, p68 and p78 (18). Under these conditions Swi2/Snf2 does not appear to make extensive contact with DNA as evident by its inability to be cross-linked to DNA. Indirect evidence suggests that the Swi2/Snf2 subunit may be able to interact with the N-terminal tails of histones H3 and H4. A bromodomain, similar to that found in the C terminus of Swi2/Snf2, has been shown *in vitro* to bind selectively to the tails of H3 and H4 but not to the tails of H2A and H2B (24).

Using a photochemical approach with site-specific modified photoreactive DNAs, we have investigated the interactions of yeast SWI/SNF with the nucleosome and present evidence for the direct interaction of Swi2/Snf2 in the SWI/SNF complex and Rsc4 in the RSC complex with DNA in a nucleosome-dependent manner. The 5 S rDNA template was chosen for these studies, because it has been well characterized to help phase the nucleosome on DNA and to cause tight rotational positioning of the nucleosome (25, 26). Although rotationally positioned, the 5 S rDNA nucleosome has more than one translational position (25, 27, 28). We have taken advantage of the several translational positions of the 5 S rDNA to scan for the interactions of SWI/SNF with nucleosomal DNA and to compare that with its interactions with naked DNA. Another important outcome of our results is the data showing significant changes in the interface between the nucleosome and the chromatin-remodeling complex after hydrolysis of ATP, which is indicative of key conformational changes of SWI/SNF or changes in the sites of DNA bound in the nucleosome.

EXPERIMENTAL PROCEDURES

Purification of SWI/SNF Complex—The SWI/SNF complex was purified from *S. cerevisiae* strain CY396 containing HA¹-tagged and His₆-tagged Swi2. The purification involved nickel-nitrilotriacetic acid agarose, DNA cellulose, MonoQ column chromatography, and glycerol gradient ultracentrifugation as described previously (29). The concentration of the SWI/SNF was determined by quantitative Western blot analysis using a polyclonal anti-Snf5 or anti-Swp73 antibody.

DNA Probe Synthesis—A 214-bp *EcoRI*-*DdeI* fragment of DNA derived from plasmid pXP-10 (30), which includes the *Xenopus borealis* somatic 5 S rRNA gene, was used to construct DNA photoaffinity probes. Each probe had photoreactive nucleotide analogs AB-dUMP (5-[N-(4-azidobenzoyl)-3-aminoallyl]dUMP) and/or AB-dCMP (4-[N-(p-azidobenzoyl)-2-aminoethyl]dCMP) incorporated adjacent to radiolabeled nucleotides at specific sites in the DNA (31). Four DNA probes, designated as 47/55, 1/5, -13/-14, and -27/-30, were synthesized as described previously (26). The numbers indicate the base pair position or range within which a modified nucleotide is incorporated with +1 being the start site of transcription. In addition, an unmodified DNA probe containing only a single radiolabeled nucleotide at bp -70 and no photoreactive nucleotides was synthesized. Probes 47/55, 1/5, and -27/-30 were on the transcribed strand, whereas probes -13/-14 and -70 were on the nontranscribed strand.

Reconstitution of Nucleosomes—Nucleosomes were reconstituted by transferring histone octamers from HeLa oligonucleosomes onto probe DNA by the octamer transfer method as described previously (26). H1-depleted oligonucleosomes from HeLa cells were prepared as described previously. Mock nucleosomal/naked DNA was prepared by carrying out octamer transfer in the absence of probe DNA. Probe DNA was added after the final step of octamer transfer.

Photoaffinity Labeling—A binding reaction was set up containing ~30–40 nM SWI/SNF and 30 nM nucleosomes in buffer A (20 mM Na-HEPES, pH 7.8, 100 mM NaCl, 3 mM MgCl₂, 0.1% Nonidet P-40, 5% glycerol, 2 mM 2-mercaptoethanol, 0.2 mM phenylmethylsulfonyl fluoride, 0.01% Tween 20, and 0.2 mg/ml bovine serum albumin). The reaction was incubated at 30 °C for 30 min. ATP, ATP_γS, ADP, AMP, GTP, CTP, and UTP were included optionally in the binding reaction at a final concentration of 100 μM. For competitor experiments, 1.8 μg (20-fold excess) of cold oligonucleosomes was added to the reaction after the binding reaction and incubated for an additional 30 min at 30 °C.

For photoaffinity labeling with RSC, the binding reactions contained 20 nM RSC and were in buffer B (20 mM K-HEPES, pH 7.8, 4 mM MgCl₂, 30 mM potassium acetate, and 75 μg/ml bovine serum albumin). ATP was included optionally in the binding reaction at a final concentration of 500 μM. The reaction was incubated at 30 °C for 30 min as before.

After the incubation, 4 μl of the binding reaction was loaded on a 4% native polyacrylamide gel in 0.5× TBE at 4 °C (acrylamide:bisacrylamide of 38.9:1.1). Alternatively, a 5 or 6% native polyacrylamide gel (acrylamide:bisacrylamide of 60:1) in 0.2× TBE was used to resolve the different translational positions of the nucleosome. Of the RSC binding reaction, 4 μl was loaded on a 3.2% native polyacrylamide gel in TE buffer (10 mM Tris-HCl, pH 7.5, and 1 mM EDTA).

The remainder of the sample was irradiated with ultraviolet light as described previously (31). The samples were treated with DNase I and S1 nuclease and analyzed on a 4–20% SDS-polyacrylamide gel. The dried gel was analyzed using a Cyclone phosphorimaging device from Packard Instruments.

Western Blot Analysis—SWI/SNF was separated on a 4–20% SDS-polyacrylamide gel. After electroblotting onto a nitrocellulose membrane and the blocking of membrane, several antibodies were used to probe the membrane (32). Antibodies to Swi3, Swp82, Swp73, Arp7, Arp9, Snf6, and Tfg3/Taf30 were provided by Bradley Cairns, and antibodies to Swi1 and Snf5 were provided by Craig Peterson. High affinity anti-HA antibody (Roche Molecular Biochemicals) was used for the detection of the Swi2/Snf2 subunit.

Restriction Endonuclease Cleavage Assay—A 50 μl-binding reaction, as described for photoaffinity labeling, was incubated at 30 °C for 30 min. Eighty units of *EcoRV* were added to each reaction followed by a 5-min incubation at 37 °C. The cleavage reaction was stopped by the addition of 10 mM EDTA. The aqueous layer was extracted first with a phenol/chloroform mixture (1:1) and then with chloroform. The DNA was precipitated by the addition of 0.1 volume of 10 M LiCl and 2.5 volumes of 100% ethanol. 10 μg of plasmid DNA was added as a carrier for the precipitation. The DNA was resuspended in 10 μl of TE containing 0.05% Tween 20 and analyzed on a 9% native polyacrylamide gel.

Immunoprecipitation—A 67-μl photoaffinity labeling reaction was incubated at 30 °C for 30 min. Four microliters of the sample was loaded on a 4% native polyacrylamide gel in 0.5× TBE. The remainder of the sample was irradiated with ultraviolet light. After irradiation, one-third of the reaction mixture was treated with DNase I and S1 nuclease as before. The remaining two-thirds of the sample were immunoprecipitated essentially as described previously (16). Protein A-Sepharose 4B resin (40 μl, Sigma) was equilibrated with buffer C (20 mM K-Hepes, pH 7.8, 10% glycerol, 12.5 mM MgCl₂, 0.1 mM EDTA, 0.2% Tween 20, 0.1 mM dithiothreitol, and 0.1 M potassium acetate). Anti-Swp73 antibody (7 μl) was bound to 20 μl of equilibrated resin for 30 min at 4 °C with mixing at 1300 rpm every 2 min. Unbound antibody was removed by washing the resin with 500 μl of buffer C.

The sample was pre-cleared by adding it to 20 μl of equilibrated resin. The supernatant was added to the antibody-bound protein A-Sepharose resin and incubated for 3 h at 4 °C with mixing as described earlier. The supernatant was removed, and the resin was washed three times with 500 μl of buffer D (20 mM K-Hepes, pH 7.8, 10% glycerol, 12.5 mM MgCl₂, 0.1 mM EDTA, 0.2% Tween 20, 0.1 mM dithiothreitol, and 0.6 M potassium acetate) and once with buffer A. The resin was resuspended in 20 μl of buffer A, and the digestion of the DNA with DNase I and S1 nuclease was carried out as described earlier. The samples were analyzed on a 4–20% SDS-polyacrylamide gel, and the dried gel was subjected to phosphorimaging.

RESULTS

The Swi2/Snf2 and Snf6 Subunits of Yeast SWI/SNF Are in Close Proximity to DNA in the SWI/SNF-Nucleosome Complex—Site-specific DNA photoaffinity labeling was used to understand how SWI/SNF interacts with the 5 S rDNA nucleosome before and after remodeling. Nucleosomes were reconstituted using a 214-bp fragment of DNA that included the *X. borealis* somatic 5 S rRNA gene. Nucleosomes reconstituted with this DNA are highly positioned rotationally and occupy several well defined translational positions (25, 27, 28). Four major dyad axes have been mapped on this DNA to base pairs -44, -24, -3 and +7 with +1 being the start site of transcription for the 5 S rRNA gene. Four photoreactive DNA probes were found that efficiently cross-linked SWI/SNF and that are distributed at varied positions in the 5 S rDNA

¹ The abbreviations used are: HA, hemagglutinin; TBE, 90 mM Tris borate, 2 mM EDTA; bp, base pair(s); ATP_γS, adenosine 5'-3-O-(thio)triphosphate; AMP-PNP, adenosine 5'-(β,γ-imino)triphosphate.

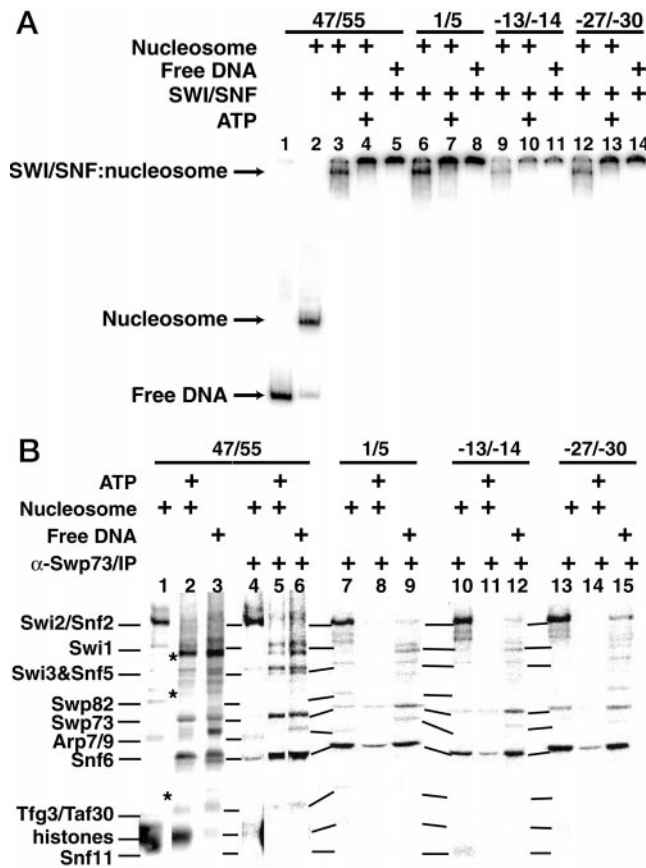


FIG. 2. The Swi2/Snf2 and Swi6 subunits of SWI/SNF are positioned near nucleosomal DNA in the SWI/SNF-nucleosome complex. *A*, gel shift analysis. Probe DNAs were reconstituted into nucleosomes by the octamer transfer method and analyzed by gel shift assay on a 4% native polyacrylamide gel in $0.5\times$ TBE. Mock nucleosome reconstitutions were performed also using the octamer transfer method, except that the probe DNA was added last after doing the final dilution of the donor nucleosomes to 100 mM NaCl (*lanes 5, 8, 11, and 14*). These samples are referred to as free DNA because no octamer transfer occurs under these conditions. The amount of SWI/SNF added in *lanes 3–14* was stoichiometric and was shown empirically by titrating with different amounts of SWI/SNF in a gel shift binding assay with a fixed amount of nucleosomes. Remodeling reactions in *lanes 4, 7, 10, and 13* contained 100 μ M ATP. *B*, DNA photoaffinity labeling. A large portion of the same samples from *A* were irradiated and digested with DNase I and S1 nuclease leaving a radiolabeled DNA tag cross-linked to protein. The samples were analyzed by 4–20% SDS-polyacrylamide gel electrophoresis and phosphorimaging analysis. The electrophoretic mobilities of the SWI/SNF subunits were determined by Western blotting using subunit-specific polyclonal antibodies and are indicated on the left. Samples in *lanes 4–15* were immunoprecipitated using an anti-SWP73 antibody before degradation of the DNA probe and subsequent analysis.

SNF, the modified DNA was cleaved to a slightly higher extent (Fig. 1, *B* and *C*, compare *lanes 4, 6, and 8*) than the unmodified nucleosome. As with unmodified DNA, the extent of cleavage increased as the amount of SWI/SNF added was increased until it reached a molar ratio of 1:3, when almost 90% of the DNA was cleaved (Fig. 1*C*, *lanes 9 and 10*). These results show that DNA modification does not interfere with the interaction of SWI/SNF with the 5 S nucleosome.

The formation of discrete SWI/SNF-nucleosome complexes was detected by gel shift assay with all four modified nucleosomes (Fig. 2*A*, *lanes 3, 6, 9, and 12*). The efficiency of nucleosome formation with all four probe DNAs was comparable with between 87 and 93% of the DNA being assembled as assayed by gel shift analysis. The amount of SWI/SNF required for quantitative binding was empirically determined by titrating SWI/SNF with a fixed amount of nucleosomal probe and assaying by

gel shift (data not shown). The addition of ATP to the SWI/SNF-nucleosome complex caused a further reduction in the electrophoretic mobility of the complex, resulting in the complex not migrating into the gel (*lanes 4, 7, 10, and 13*). The binding of SWI/SNF to free DNA also formed a complex that does not migrate into the gel (*lanes 5, 8, 11, and 14*). The electrophoretic properties of the SWI/SNF:DNA complex suggest that several SWI/SNF molecules may be bound per DNA. The SWI/SNF-nucleosome complexes were photocross-linked at the four different regions in the nucleosome by irradiating the complexes and then digesting away most of the cross-linked DNA with DNase I and S1 nuclease. The short labeled DNA fragment left covalently attached to either histones or SWI/SNF serves as a radioactive tag to identify the components of the complex cross-linked at the various sites on the 5 S rDNA. DNA probe 47/55 most efficiently cross-linked histones and a \sim 205-kDa protein (Fig. 2*B*, *lane 1*). The electrophoretic mobility of the 205-kDa protein was shown to be similar to that of the Swi2/Snf2 protein by Western blotting analysis with anti-HA antibody detection of the Swi2/Snf2-HA-His₆ (data not shown). Minor cross-linking of several other proteins was also observed. These polypeptides with apparent molecular masses of 127, 92, 76, 63, and 50 kDa were found by Western blot analysis to have electrophoretic mobilities similar to that of Swi3/Snf5, Swp82, Swp73, Arp7/Arp9, and Snf6, respectively.

Cross-linking of the 205-kDa polypeptide was dramatically reduced by the addition of ATP (Fig. 2*B*, *lane 2*). These results indicate that the Swi2/Snf2 subunit is located close to nucleosomal DNA upon binding to the nucleosome but after chromatin remodeling is displaced from the nucleosome so that it cannot be cross-linked to DNA. In the experiment with cross-linking of SWI/SNF bound to naked DNA, the Swi2/Snf2 subunit is not cross-linked at base pairs 47/55 in the 5 S rDNA (*lane 3*). Thus, these data indicate that the association of Swi2/Snf2 near DNA is nucleosome-dependent. Proteins with relative mobilities similar to that of Swi1, Arp7 or Arp9, and Snf6 are most readily cross-linked in the SWI/SNF bound to naked DNA in the absence of ATP. Similarly, these same proteins are cross-linked when SWI/SNF is associated with the nucleosome in the presence of ATP, with the exception of the \sim 60- and \sim 63-kDa protein (Arp7 and Arp9). Other positions on the 5 S rDNA, some near the ends of the DNA, were also probed but did not efficiently cross-link SWI/SNF (data not shown).

The photoaffinity-labeled SWI/SNF complex was purified away from labeled histones and other proteins by immunoprecipitation using an anti-Swp73 antibody. Swp73 is a highly conserved subunit of SWI/SNF that is tightly associated with the complex, thus making it possible to immunoprecipitate complete SWI/SNF complexes (33). SWI/SNF is dissociated from the nucleosome under the salt concentrations used in the immunoprecipitation. After extensive washing of the beads with SWI/SNF still bound to the protein A-Sepharose, the sample is digested with DNase I and S1 nuclease, and the protein is eluted by adding a sample loading buffer. Six of the proteins cross-linked with probe 47/55 were immunoprecipitated and correspond by relative molecular mass to the Swi2/Snf2, Swi3, Swp82, Swp73, Arp7/Arp9, and Snf6 subunits of the SWI/SNF complex (Fig. 2*B*, *lane 4*). Swi2/Snf2 was the most intensely cross-linked subunit, suggesting again that it makes close contact with nucleosomal DNA. The 161-, 103-, and 35-kDa polypeptides (compare *lane 1* with *lane 4*, *) did not immunoprecipitate and are probably contaminants present in SWI/SNF. The histone proteins were also removed by immunoprecipitation and facilitated in identifying whether the Snf11 subunit of SWI/SNF was cross-linked.

Immunoprecipitation of the SWI/SNF-nucleosome complex

after remodeling showed that the interactions of SWI/SNF with the nucleosome were greatly altered (*lane 5*). The Swi2/Snf2 subunit was no longer cross-linked efficiently, suggesting that it does not make close contact with DNA at this position in the remodeled nucleosome. The most efficiently cross-linked subunit of SWI/SNF after remodeling is Snf6. Although much less efficiently cross-linked in the initial complex, the contacts of the Swp82 and Arp7 or Arp9 subunits were also lost upon the addition of ATP. On the other hand, the contacts of Swp73 and Snf6 were increased, and in addition, Swi1, Snf5, and Tfg3/Taf30 were cross-linked to DNA, although weakly only after the addition of ATP. These altered contacts of SWI/SNF with the nucleosome after remodeling bear great similarity to the interaction of SWI/SNF with naked DNA (*compare lane 5 with lane 6*).

To examine SWI/SNF contacts at other positions across the nucleosome, several other probes were synthesized. Three probes, 1/5, -13/-14, and -27/-30, cross-linked the Swi2/Snf2 subunit of SWI/SNF in a nucleosome-specific manner. Immunoprecipitation of the SWI/SNF subunits cross-linked to nucleosomal probes 1/5, -13/-14, and -27/-30 showed that Swi2/Snf2 and Snf6 were efficiently cross-linked to DNA (Fig. 2B, *lanes 7, 10, and 13*). The cross-linking of Swi2/Snf2 was the most dramatically decreased in the presence of ATP as with probe 47/55 (*compare lanes 7, 10, and 13 with lanes 8, 11, and 14*). Minor cross-linking of other subunits, including Swi1, Swi3/Snf5, Swp82, Swp73, Arp9 and Tfg3/Taf30, was also obtained. The two weak bands immediately below the Swi2/Snf2 subunit are probably degradation products of Swi2/Snf2 and varied in intensity in different preparations of SWI/SNF. Snf6 appears to interact closely with free DNA, because it is cross-linked efficiently when SWI/SNF is bound to DNA alone (*lanes 9, 12, and 15*). At these three positions, the efficiency of Snf6 cross-linking does decrease with the addition of ATP. Swp73 also interacts with nucleosomal and naked DNA but to a lesser extent as compared with Snf6.

Hydrolysis and Not Just Binding of ATP Is Required for Changes in the Interface between SWI/SNF and the Nucleosome—To ascertain if the binding of ATP alone or hydrolysis of the γ -phosphate of ATP was required for the loss of photoaffinity labeling of the Swi2/Snf2 subunit or changes in the SWI/SNF-nucleosome complex, several ATP analogs as well as other nucleotide triphosphates were used. As before, the addition of ATP resulted in a change in the gel-shifted complex, which no longer entered into the gel (Fig. 3A, *lanes 4 and 5*). ATP γ S and AMP-PNP did not change the electrophoretic mobility of the initial SWI/SNF-nucleosome complex (*lanes 6 and 7*). Similarly, ADP, AMP, and other nucleotide triphosphates did not change the mobility of the complex (data not shown). Consistent with the observation that a hydrolyzable form of ATP was required for significant changes in the electrophoretic mobility of the SWI/SNF-nucleosome complex, a loss in DNA photoaffinity labeling of the Swi2/Snf2 protein with probe 47/55 was also observed to require hydrolysis of the γ -phosphate of ATP (Fig. 3B). In the presence of ATP, there was a decrease in histone-DNA contacts and a loss of Swi2/Snf2 cross-linking as well as an increase in cross-linking Swp73 and Snf6 subunits of SWI/SNF (Fig. 3B, *lanes 2 and 3*). However, there was no change in the efficient cross-linking of Swi2/Snf2 with ATP γ S, ADP, AMP, GTP, CTP, or UTP (*lanes 4–9*). The requirement for the hydrolysis of the γ -phosphate of ATP for loss of Swi2/Snf2 cross-linking was also observed with probes 1/5, -13/-14, and -27/-30 (data not shown).

Altered Conformation of the Nucleosome after Remodeling Is Detected by Gel Shift Assay—Because the cross-linking of SWI/SNF to the nucleosome after the addition of ATP resembles

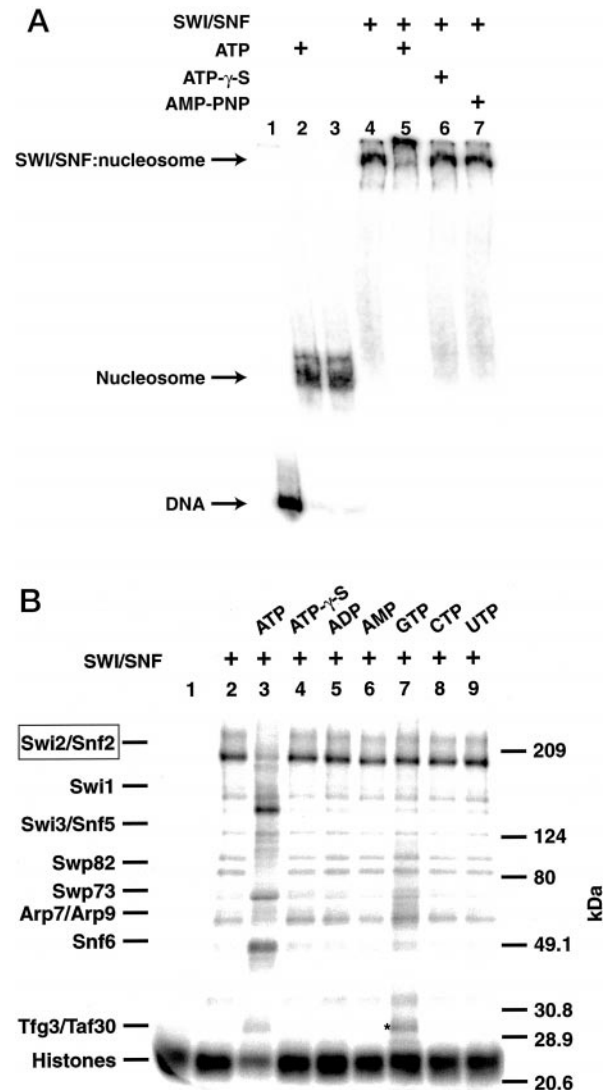


FIG. 3. Loss of Swi2/Snf2 cross-linking and change of the electrophoretic mobility of the SWI/SNF-nucleosome requires hydrolysis of the γ -phosphate of ATP. A, gel shift analysis of the SWI/SNF-nucleosome complex with DNA probe 47/55 in the presence of ATP (*lane 5*), ATP γ S (*lane 6*), and AMP-PNP (*lane 7*). *Lane 1* is the DNA alone, and *lanes 2 and 3* are the 5 S nucleosome alone with and without ATP. B, DNA photoaffinity labeling. Nucleosomes reconstituted with probe 47/55 were bound to SWI/SNF in the presence of ATP (*lane 3*), ATP γ S (*lane 4*), ADP (*lane 5*), AMP (*lane 6*) or other nucleotide triphosphates (*lanes 7–9*). After cross-linking, the samples were analyzed by 4–20% SDS-polyacrylamide gel electrophoresis. The band marked with an asterisk (*) in *lane 7* was not reproducible and was probably caused by incomplete digestion of DNA.

that of SWI/SNF bound to DNA alone, it was important to determine whether the probe DNA was still bound to the nucleosome after the remodeling reaction. After remodeling, a change in the nucleosome complexes was observed by high-resolution gel shift assay after competing SWI/SNF away with an excess of competitor nucleosomes (Fig. 4A, *compare lanes 2 and 6*). The initial 5 S rDNA nucleosome complex has three distinct forms that can be resolved by gel electrophoresis (*lane 2*). Previously, a centrally located nucleosome had been observed to have a lower electrophoretic mobility than one located closer to the ends of DNA (34, 35). After remodeling there was still some of the N1 complex, but there was a distinct loss of the more acentrally positioned nucleosomes (N2 and N3) (*lane 6*). An increased smear in the lane and the generation of additional free DNA were observed after remodeling. The total amount of free DNA generated after remodeling was only approximately

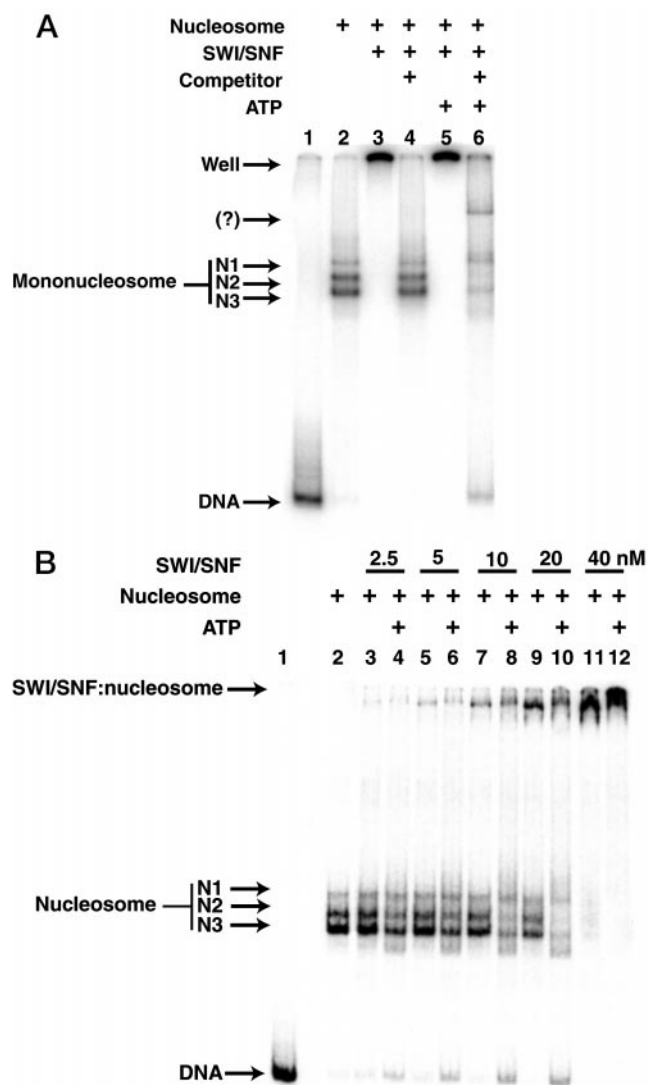


FIG. 4. Altered conformation of the nucleosome after remodeling as detected by gel shift assay. *A*, a stoichiometric amount of SWI/SNF was used in lanes 3–6. SWI/SNF was competed away from the radiolabeled 5 S nucleosome by the addition of a 20-fold excess of cold oligonucleosomes before and after remodeling (lanes 4 and 6, respectively). The samples were analyzed by native 5% polyacrylamide gel electrophoresis (0.2× TBE) at 4 °C. The nucleosome occupies at least three different translational positions with N1 and N3 being the most centric and acentric translational positions, respectively. *B*, the amount of SWI/SNF was varied while maintaining a constant amount of nucleosome to determine the amount of SWI/SNF required to see changes in the 5 S nucleosome with no competitor added. The concentrations of SWI/SNF are indicated above each lane, and lanes 4, 6, 8, 10, and 12 all contained 100 μ M ATP.

10% after taking into account the original amount of free DNA present in the nucleosome assembly. Thus, most of the DNA in the starting nucleosome core particle is still bound but not in a discrete nucleosome complex. The binding of SWI/SNF to the nucleosome alone was not sufficient to alter the nucleosome complex (lane 4). After remodeling, the band migrating higher than the original N1 complex migrates similarly to the reported dinucleosome reported for yeast RSC and human SWI/SNF (36, 37). This complex was not consistently observed in our experiments.

The loss of discrete nucleosome complexes was assayed at different ratios of SWI/SNF to nucleosome to determine whether this same change could be detected at substoichiomet-

ric amounts of SWI/SNF (Fig. 4*B*). At less than stoichiometric amounts of SWI/SNF and without competitor, a loss of discrete nucleosome complexes and the release of some DNA from the nucleosome were detected upon remodeling (compare lanes 3, 5, 7, and 9 with lanes 4, 6, 8, and 10). These results show that less than stoichiometric amounts of SWI/SNF are sufficient to cause a loss of discrete nucleosome complex and enhance the release of DNA from the nucleosome.

The Interactions of RSC with a 5 S Nucleosome Differ from Those of SWI/SNF—RSC formed a discrete complex with nucleosomal and naked DNA (Fig. 5*A*, lanes 2 and 5). In the presence of ATP, the electrophoretic mobility of the RSC-nucleosome complex was unchanged under those gel conditions (lane 3). Photoaffinity labeling with probe 47/55 revealed that four subunits of the RSC complex were cross-linked to the nucleosomal DNA (Fig. 5*B*, lane 2), and these included Sth1 (the Swi2/Snf2 homolog), Rsc3, and Rsc4, with minor cross-linking of Rsc2. Unlike the Swi2/Snf2 subunit of the SWI/SNF complex, Sth1 did not appear to have a specificity for nucleosomal DNA and was cross-linked equally efficiently to naked DNA (compare lane 2 with lane 5). The only subunit that was cross-linked in a nucleosome-specific manner was Rsc4.

As with the SWI/SNF complex, the cross-linking of RSC after the addition of ATP strongly resembled that obtained with naked DNA. In the presence of ATP, the cross-linking of Sth1 and Rsc3 was unchanged; however, the cross-linking of the Rsc2 subunit was increased, whereas that of Rsc4 was eliminated (lane 3). Another subunit of RSC was also cross-linked to the remodeled nucleosome complex or the RSC bound to DNA alone, and it is one of the Rsc5–10 or Sfh1 subunits, but it could not be uniquely identified because of the inadequate separation of these subunits.

DISCUSSION

We have focused on examining the interface between the chromatin-remodeling complexes SWI/SNF or RSC, and the nucleosome by 1) identifying which subunits of SWI/SNF or RSC are near the nucleosomal DNA, 2) comparing or contrasting these interactions with how SWI/SNF and RSC bind naked DNA, and 3) determining how contacts with the nucleosome change after the remodeling reaction. Our approach has been to attach photoreactive side chains to specific sites on a DNA, which has intrinsic nucleosome-positioning properties. Different *X. borealis* 5 S rDNA probes were synthesized and assembled into nucleosomes to investigate regions facing both in toward the octamer and away from the octamer. Each of these DNA probes has several positions on the octamer, because each is bound to the nucleosome in one of several translational positions.

Site-specific DNA-protein cross-linking has shown that the Swi2/Snf2 and Snf6 subunits of the yeast SWI/SNF complex are in close proximity to nucleosomal DNA in the SWI/SNF-nucleosome complex. SWI/SNF was shown to bind to the nucleosome and form a discrete complex by gel shift or electrophoretic mobility shift assay under conditions that were also optimal for cross-linking the 205- and 50-kDa proteins. The photoaffinity-labeled 205- and 50-kDa polypeptides were shown to be the Swi2/Snf2 and Snf6 subunits of SWI/SNF by comparing the electrophoretic mobility of the labeled proteins with that of the SWI/SNF subunits detected by immunoblotting with subunit-specific polyclonal antibodies. These labeled proteins were further shown to be part of the SWI/SNF complex by immunoprecipitation with anti-Swp73 antibodies in a manner similar to that originally used by others to purify SWI/SNF (16, 33). The Swi2/Snf2 subunit is consistently the most or one of the most readily cross-linked subunits regardless of where the

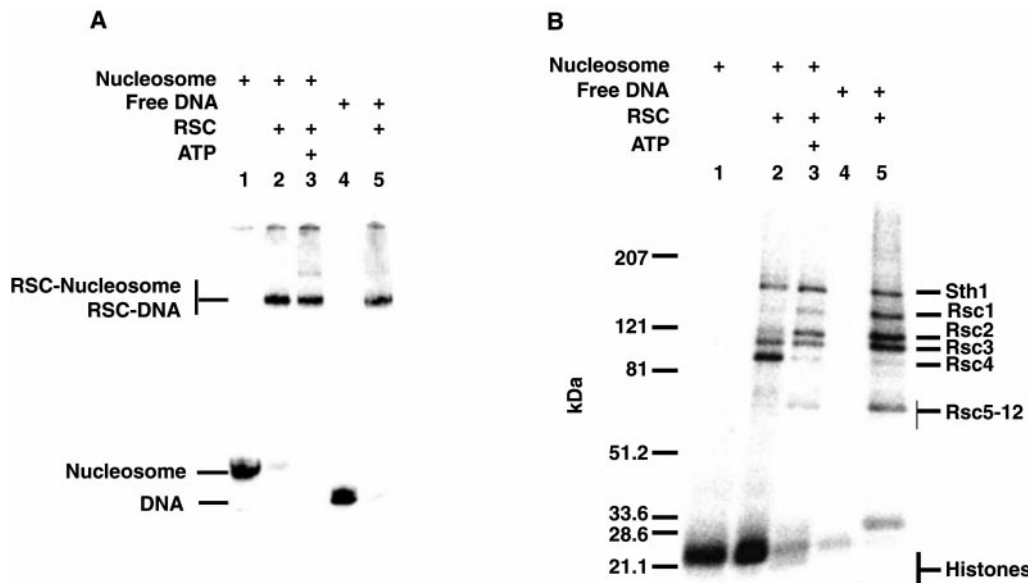


FIG. 5. The interactions of the RSC complex with the 5 S rDNA nucleosome differ from those of SWI/SNF. *A*, Gel shift analysis. 5 S rDNA nucleosomes assembled with probe 47/55 (lanes 1–3) or free DNA (lanes 4–5) were bound to RSC either in the presence (lane 3) and/or absence of 500 μ M ATP. The complexes were analyzed on a 3.2% native polyacrylamide gel in 10 mM Tris-HCl, pH 7.5, and 1 mM EDTA. Free DNA samples were prepared the same as described in Fig. 2. *B*, DNA photoaffinity labeling. A portion of each sample from *A* was irradiated, enzymatically digested, and analyzed by 4–20% SDS-polyacrylamide gel electrophoresis and phosphorimaging analysis. The pre-stained molecular mass standards are as indicated on the right.

nucleosome is probed. The Snf6 subunit is also one of the most efficiently cross-linked subunits of SWI/SNF with three of the four different DNA probes used in this study. Several other SWI/SNF subunits were cross-linked with a low efficiency, suggesting that they are not as closely associated with nucleosomal DNA as are Swi2/Snf2 and Snf6.

The Swi2/Snf2 and Snf6 subunits, thus presumably, play an important role in the chromatin-remodeling reaction because of their position in the interface between the chromatin-remodeling complex and the nucleosome. Although this is the first evidence of direct interaction between the Swi2/Snf2 subunit and nucleosomal DNA, other lines of evidence have shown that Swi2/Snf2 or its homolog is the catalytic subunit of ATP-dependent chromatin-remodeling complexes and as such might be expected to interact directly with the nucleosome. Mutational analysis of Swi2/Snf2 has shown that the elimination of the ATPase activity of Swi2/Snf2 inactivates the remodeling activity of SWI/SNF (13, 17). Recombinant human Swi2/Snf2 homologs, hBrm and Brg1, have been shown alone to have minimal remodeling activity that is stimulated by the presence of three other subunits of the human SWI/SNF complex related to the Swi3 and Snf5 subunits of yeast SWI/SNF (23). Also, the recombinant *Drosophila* protein ISWI, a Swi2/Snf2 homolog, has been shown alone to have comparable levels of remodeling activity with that of the NURF, CHRAC, and ACF complexes that contain ISWI (9). These results indicate that the catalytic center of the ATP-dependent chromatin-remodeling complexes resides in the Swi2/Snf2-like subunit, and now we present evidence that the catalytic center containing the subunit contacts the nucleosome.

Less is known about the Snf6 subunit, an essential subunit of SWI/SNF, than the Swi2/Snf2 subunit and there is no known homolog in other chromatin-remodeling complexes (38). The C terminus, containing an acidic region and a glutamine-rich region, is not absolutely required *in vivo* for SWI/SNF activity, and the extreme N terminus contains a highly basic region (39). The association of Snf6, unlike Swi2/Snf2, with DNA is not dependent on the packaging of DNA into a nucleosome core

particle. Snf6 is cross-linked efficiently by all four DNA probes when SWI/SNF is bound to DNA alone. Therefore, Snf6 presumably has a general affinity for DNA as assembled in the SWI/SNF complex and may help promote SWI/SNF binding to free DNA or to nucleosomal DNA.

It is significant that Swi2/Snf2 only cross-links well to nucleosomal DNA and not to free DNA. The cross-linking data indicate that the histone octamer helps recruit the Swi2/Snf2 subunit to the surface of the DNA or stabilizes its interaction with DNA in the SWI/SNF-nucleosome complex. Sequence analysis of SWI2/SNF2 reveals a bromodomain located at the C terminus, and a similar bromodomain in GCN5 and TAF₁₁250 has been indicated to bind to the N-terminal tails of histones H3 and H4 (24, 40). An interaction between the bromodomain of Swi2/Snf2 and histones H3 and H4 would be consistent with the nucleosome-specific cross-linking of Swi2/Snf2 observed with all four DNA photoaffinity probes. Although the bromodomain may facilitate in the interaction of SWI/SNF with the nucleosome, it is not essential *in vivo* for its chromatin-remodeling activity as shown by Laurent and Carlson (38). SWI/SNF and RSC can also remodel trypsinized histone octamers, suggesting that tail interactions are not essential for remodeling (41, 42).

The cross-linking data suggest that the Swi2/Snf2 subunit is displaced out of the interface between the nucleosome and SWI/SNF after remodeling and is therefore not able to be cross-linked to DNA after the addition of ATP. The conformational change observed by both changes in DNA photoaffinity labeling and gel shift analysis required the hydrolysis of the γ -phosphate of ATP and did not occur by binding ATP alone or with other ribonucleotide triphosphates. There is a significant similarity in both the gel shift and DNA cross-linking of the SWI/SNF-nucleosome complex after remodeling with that of SWI/SNF bound to free DNA. These similarities are consistent with the nucleosome structure being altered so significantly that SWI/SNF can bind to the complex essentially as it does to free DNA. It is clear from the gel shift data that although some of the octamer have been displaced, most

had not been displaced from the DNA probe. The competition of SWI/SNF away from the remodeled nucleosomes demonstrates that most of the DNA are still bound to the octamer and that the nucleosome after remodeling is changed sufficiently to alter its electrophoretic mobility. Changes in the electrophoretic mobility of the nucleosome could be caused by deformation of the nucleosome or by sliding of the octamer on DNA to many different translational positions. It is not possible to differentiate between these two possibilities. Other data have suggested that SWI/SNF can slide the nucleosome along DNA similar to that of other chromatin remodeling complexes, such as NURF and CHRAC (21, 22, 43, 44). Sliding mediated by ISWI complexes of mononucleosome substrates has shown sliding to a preferred translational position. Similarly, the yeast ISW2 complex preferentially slides the nucleosome on the same 5 S rDNA probes used in this report to a unique more centric translational position.² These sliding results are noticeably different from that observed for SWI/SNF in that there is no preferred translational position but rather a diffuse smear of many potentially alternative translational positions. Although nucleosome sliding could be the cause of the reduced Swi2/Snf2 cross-linking, it would be expected that at some positions on DNA, there should be enhanced cross-linking of Swi2/Snf2 after remodeling. The data however indicate that the loss of Swi2/Snf2 most probably is not attributed primarily to nucleosome sliding, because at no position probed is there an increase in the cross-linking of Swi2/Snf2. There is no increase in Swi2/Snf2 cross-linking even near the ends of the DNA (data not shown).

We have compared the interactions of the RSC complex with the 5 S nucleosome to those of the SWI/SNF complex. The Sth1 protein has a 72% identity over 661 amino acids to the Swi2/Snf2 protein and could be expected to interact with the nucleosome in a manner similar to that of Swi2/Snf2 (45, 46). However, unlike Swi2/Snf2, Sth1 is cross-linked to DNA both in the DNA alone bound to RSC and in the RSC bound to the nucleosome. The Sth1 subunit also differs from Swi2/Snf2, because it is not displaced from the nucleosomal DNA after remodeling. Both Sth1 and Swi2/Snf2 proteins contain bromodomains at the C terminus and potentially could interact directly with the nucleosome. The cross-linking demonstrates that Rsc4 is specifically recruited by the nucleosome to be near the DNA, as is Swi2/Snf2 in the SWI/SNF complex. Rsc4 has been characterized as a protein that may interact with histone tails,³ consistent with the observed nucleosome-specific cross-linking. Rsc4 and Swi2/Snf2 behave similarly in that both are displaced from the surface of DNA after remodeling. The key difference between the mode of action for SWI/SNF and that of RSC is that only in SWI/SNF is the catalytic center apparently displaced from DNA. This potential difference in their mode of action is also suggested in genetic studies showing that SWI/SNF has different interactions with chromatin than RSC. Mutations in histone and nonhistone proteins that suppress *swi1/snf* defects were found to enhance the defects of temperature-sensitive mutants of *sth1* (47).

Next, the Rsc2 subunit apparently takes the place of Rsc4 near DNA after remodeling, because Rsc2 is only cross-linked to DNA in the RSC-nucleosome complex after the addition of ATP. When RSC binds to naked DNA, the Rsc1 and Rsc2 subunits are also efficiently cross-linked. Rsc1 and Rsc2 contain an AT hook domain that has been associated with DNA binding activity and could account for why these two subunits make close contact with DNA when RSC is bound to DNA alone (48).

The interface between the chromatin-remodeling complexes (SWI/SNF and RSC) and the nucleosome core particle has been probed using site-specific DNA photoaffinity labeling. Not only were some of the subunits of these complexes shown to be located at this interface, but also these interactions changed upon hydrolysis of ATP. In the future, it will be important to use this approach to examine changes in the nucleosome core particle after remodeling to better understand the structure of the "remodeled" nucleosome.

Acknowledgments—We thank Stefan Kassabov and Martin Zofall for helpful discussions and comments regarding this work.

REFERENCES

- Grant, P. A., and Berger, S. L. (1999) *Semin. Cell Dev. Biol.* **10**, 169–177
- Ayer, D. E. (1999) *Trends Cell Biol.* **9**, 193–198
- Kuo, M. H., and Allis, C. D. (1998) *Bioessays* **20**, 615–626
- Brown, C. E., Lechner, T., Howe, L., and Workman, J. L. (2000) *Trends Biochem. Sci.* **25**, 15–19
- Tong, J. K., Hassig, C. A., Schnitzler, G. R., Kingston, R. E., and Schreiber, S. L. (1998) *Nature* **395**, 917–921
- Xue, Y., Wong, J., Moreno, G. T., Young, M. K., Cote, J., and Wang, W. (1998) *Mol. Cell* **2**, 851–861
- Zhang, Y., Ng, H. H., Erdjument-Bromage, H., Tempst, P., Bird, A., and Reinberg, D. (1999) *Genes Dev.* **13**, 1924–1935
- Wade, P. A., Jones, P. L., Vermaak, D., and Wolffe, A. P. (1998) *Curr. Biol.* **8**, 843–846
- Corona, D. F., Langst, G., Clapier, C. R., Bonte, E. J., Ferrari, S., Tamkun, J. W., and Becker, P. B. (1999) *Mol. Cell* **3**, 239–245
- Tsukiyama, T., Palmer, J., Landel, C. C., Shiloach, J., and Wu, C. (1999) *Genes Dev.* **13**, 686–697
- Cairns, B. R., Lorch, Y., Li, Y., Zhang, M., Lacomis, L., Erdjument-Bromage, H., Tempst, P., Du, J., Laurent, B., and Kornberg, R. D. (1996) *Cell* **87**, 1249–1260
- Kwon, H., Imbalzano, A. N., Khavari, P. A., Kingston, R. E., and Green, M. R. (1994) *Nature* **370**, 477–481
- Cote, J., Quinn, J., Workman, J. L., and Peterson, C. L. (1994) *Science* **265**, 53–60
- Kingston, R. E., and Narlikar, G. J. (1999) *Genes Dev.* **13**, 2339–2352
- Peterson, C. L., and Workman, J. L. (2000) *Curr. Opin. Genet. Dev.* **10**, 187–192
- Cairns, B. R., Kim, Y. J., Sayre, M. H., Laurent, B. C., and Kornberg, R. D. (1994) *Proc. Natl. Acad. Sci. U. S. A.* **91**, 1950–1954
- Richmond, E., and Peterson, C. L. (1996) *Nucleic Acids Res.* **24**, 3685–3692
- Quinn, J., Fyrberg, A. M., Ganster, R. W., Schmidt, M. C., and Peterson, C. L. (1996) *Nature* **379**, 844–847
- Peterson, C. L., Zhao, Y., and Chait, B. T. (1998) *J. Biol. Chem.* **273**, 23641–23644
- Cairns, B. R., Erdjument-Bromage, H., Tempst, P., Winston, F., and Kornberg, R. D. (1998) *Mol. Cell* **2**, 639–651
- Langst, G., Bonte, E. J., Corona, D. F., and Becker, P. B. (1999) *Cell* **97**, 843–852
- Hamiche, A., Sandaltzopoulos, R., Gdula, D. A., and Wu, C. (1999) *Cell* **97**, 833–842
- Phelan, M. L., Sif, S., Narlikar, G. J., and Kingston, R. E. (1999) *Mol. Cell* **3**, 247–253
- Ornaghi, P., Ballario, P., Lena, A. M., Gonzalez, A., and Filetici, P. (1999) *J. Mol. Biol.* **287**, 1–7
- Pruss, D., Bartholomew, B., Persinger, J., Hayes, J., Arents, G., Moudrianakis, E. N., and Wolffe, A. P. (1996) *Science* **274**, 614–617
- Sengupta, S. M., Persinger, J., Bartholomew, B., and Peterson, C. L. (1999) *Methods* **19**, 434–446
- Hayes, J. J., Tullius, T. D., and Wolffe, A. P. (1990) *Proc. Natl. Acad. Sci. U. S. A.* **87**, 7405–7409
- Panetta, G., Buttinelli, M., Flaus, A., Richmond, T. J., and Rhodes, D. (1998) *J. Mol. Biol.* **282**, 683–697
- Logie, C., and Peterson, C. L. (1999) *Methods Enzymol.* **304**, 726–741
- Wolffe, A. P., and Brown, D. D. (1986) *Cell* **47**, 217–227
- Lannutti, B. J., Persinger, J., and Bartholomew, B. (1996) *Biochemistry* **35**, 9821–9831
- Renart, J., and Sandoval, I. V. (1984) *Methods Enzymol.* **104**, 455–460
- Cairns, B. R., Levinson, R. S., Yamamoto, K. R., and Kornberg, R. D. (1996) *Genes Dev.* **10**, 2131–2144
- Meersseman, G., Pennings, S., and Bradbury, E. M. (1992) *EMBO J.* **11**, 2951–2959
- Dong, F., Hansen, J. C., and van Holde, K. E. (1990) *Proc. Natl. Acad. Sci. U. S. A.* **87**, 5724–5728
- Lorch, Y., Cairns, B. R., Zhang, M., and Kornberg, R. D. (1998) *Cell* **94**, 29–34
- Schnitzler, G., Sif, S., and Kingston, R. E. (1998) *Cell* **94**, 17–27
- Laurent, B. C., and Carlson, M. (1992) *Genes Dev.* **6**, 1707–1715
- Estruch, F., and Carlson, M. (1990) *Mol. Cell Biol.* **10**, 2544–2553
- Jacobson, R. H., Ladurner, A. G., King, D. S., and Tjian, R. (2000) *Science* **288**, 1422–1425
- Guyon, J. R., Narlikar, G. J., Sif, S., and Kingston, R. E. (1999) *Mol. Cell Biol.* **19**, 2088–2097
- Logie, C., Tse, C., Hansen, J. C., and Peterson, C. L. (1999) *Biochemistry* **38**, 2514–2522
- Jaskelioff, M., Gavin, I. M., Peterson, C. L., and Logie, C. (2000) *Mol. Cell Biol.* **20**, 3058–3068

² N. Henry and B. Bartholomew, personal communication.

³ H. Szerlong and B. Cairns, unpublished data.

44. Whitehouse, I., Flaus, A., Cairns, B. R., White, M. F., Workman, J. L., and Owen-Hughes, T. (1999) *Nature* **400**, 784–787
45. Laurent, B. C., Yang, X., and Carlson, M. (1992) *Mol. Cell. Biol.* **12**, 1893–1902
46. Tsuchiya, E., Uno, M., Kiguchi, A., Masuoka, K., Kanemori, Y., Okabe, S., and Mikayawa, T. (1992) *EMBO J.* **11**, 4017–4026
47. Du, J., Nasir, I., Benton, B. K., Kladde, M. P., and Laurent, B. C. (1998) *Genetics* **150**, 987–1005
48. Cairns, B. R., Schlichter, A., Erdjument-Bromage, H., Tempst, P., Kornberg, R. D., and Winston, F. (1999) *Mol. Cell* **4**, 715–723
49. Hayes, J. J., Clark, D. J., and Wolffe, A. P. (1991) *Proc. Natl. Acad. Sci. U. S. A.* **88**, 6829–6833