Accelerated Publication

THE JOURNAL OF BIOLOGICAL CHEMISTRY
Vol. 275, No. 20, Issue of May 19, pp. 14787–14790, 2000

Purification and Characterization of a Human Factor That Assembles and Remodels Chromatin*

Received for publication, February 11, 2000, and in revised form, March 15, 2000
Published, JBC Papers in Press, March 28, 2000, DOI 10.1074/jbc.C000093200

Gary LeRoy‡, Alejandra Loyola‡, William S. Lane§, and Danny Reinberg‡¶

From the ‡Howard Hughes Medical Institute, Division of Nucleic Acid Enzymology, Department of Biochemistry, Robert Wood Johnson Medical School, Piscataway, New Jersey 08854 and the §Harvard Microchemistry Facility, Harvard University, Cambridge, Massachusetts 02138

We have previously reported the isolation and characterization of a nucleosome remodeling and spacing factor, RSF. One of the RSF subunits is hSNF2h, a SNF2 homologue. Here we set out to isolate and characterize other hSNF2h-containing complexes. We have identified a novel hSNF2h complex that facilitates ATP-dependent chromatin assembly with the histone chaperone NAP-1. The complex possesses ATPase activity that is DNA-dependent and nucleosome-stimulated. This complex is capable of facilitating ATP-dependent nucleosome remodeling and transcription initiation from chromatin templates. In addition to hSNF2h, this complex also contains a 190-kDa protein encoded by the *BAZ1A* gene. Since both subunits are homologues of the *Drosophila* ACF complex (ATP-utilizing chromatin assembly and remodeling factor), we have named this factor human ACF or hACF.

In eucaryotic cells, the DNA is packaged with histones in the form of chromatin (1). The primary unit of chromatin is the nucleosome, composed of 147 bp[1] of DNA wrapped around an octamer of histone proteins (2). In general, the majority of nucleosomes throughout the genome are evenly spaced with a repeat length of ~190 bp (3); however, variations in repeat lengths at particular genes have been observed (4). Nucleosomes within arrays stack along with accessory proteins to form higher order structures (5). The packaging of DNA into chromatin allows for efficient storage of genetic information, although this compaction impedes the interaction of proteins with DNA. In the nucleus there are machinery's that facilitate the mobility of nucleosomes and aid proteins to bind to their sites on DNA (6, 7).

Through genetic and biochemical studies several of these enzymes have been identified from different organisms. They include the SWI/SNF complexes in yeast, human, and *Drosophila*; the RSC complex in yeast; the RSF complex in human; the Mi-2/NuRD complexes in human and *Xenopus*; the ACF, CHRAC, and NURF complexes in *Drosophila;* and the *ISWI1* and *ISWI2* complexes in yeast. A common property of these enzymes is that they utilize ATP to facilitate the mobility and/or alter the structure of nucleosomes. All of these enzymes contain a subunit with an ATPase/helicase domain with significant homology to yeast SNF2 (6–9). *In vitro*, it has been demonstrated that many of these enzymes are capable of facilitating a local perturbation of chromatin structure near the binding sites for site-specific factors, which is dependent on the binding of the site-specific factors and ATP. This phenomenon is referred to as "nucleosome-remodeling." Nucleosome-remodeling surrounding factor binding sites explains why "nuclease-hypersensitive" sites are found in the enhancer and promoter regions of active genes (10). *In vitro* studies have also demonstrated that nucleosome-remodeling enzymes can participate in transcription from chromatin templates (11–14). Recent studies have shown that while some of these enzymes can be recruited by directly interacting with the site-specific binding factors, others may function by altering the mobility of nucleosomes (7, 15–17). The ability to alter nucleosome mobility explains why some of these enzymes are capable of spacing nucleosomes. It has been demonstrated that the *Drosophila* CHRAC and ACF complexes, human RSF complex, and yeast *ISWI* complexes are capable of converting irregularly spaced chromatin into arrays of periodically spaced nucleosomes. In combination with the histone chaperone NAP-1, which deposits histones onto DNA in an ATP-independent manner, these enzymes can assemble chromatin that resembles physiologic conditions (8, 11, 12, 18).

We have previously reported the development of a minimal defined system capable of transcribing chromatin templates (11, 19). This system requires a nucleosome-remodeling enzyme to facilitate factor binding and transcription initiation. The nucleosome-remodeling factor that we used was RSF, an enzyme possessing ATP-dependent nucleosome remodeling and spacing activities. RSF is composed of two subunits, hSNF2h (135 kDa) and a 325-kDa polypeptide. This system also requires a chromatin-specific elongation factor, FACT (facilitates chromatin transcription) for RNA polymerase II to traverse nucleosomes during elongation. FACT is a two-subunit factor composed of a human homologue of the *Saccharomyces cerevisiae* Spt16/Cdc68 protein and the HMG-1-like protein SSRP1. FACT does not require ATP for energy and is thought to function as a histone chaperone (19). Here we report the isolation of a novel hSNF2h complex, distinct from RSF. This complex also contains a 190-kDa protein encoded by *BAZ1A*, a recently identified gene with similarity to the *WSTF* gene, a gene deleted in Williams Syndrome (20, 21). *BAZ1A* stands for bromodomain adjacent to zinc finger domain1A. BAZ1A and WSTF are human homologues of Acf1 the 185-kDa subunit of the *Drosophila* ACF complex, which also contains *Drosophila* ISWI (22). We therefore named this complex human ACF (hACF). Similar to *Drosophila* ACF and human RSF, the hACF complex also possesses ATP-dependent nucleosome

* This work was supported by National Institutes of Health Grant GM37120 and the Howard Hughes Medical Institute. 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.

¶ To whom correspondence should be addressed. Tel.: 732-235-4195; Fax: 732-235-5294; E-mail: reinbedf@umdnj.edu.

[1] The abbreviations used are: bp, base pair(s); RSF, remodeling and spacing factor; ACF, ATP-utilizing chromatin assembly and remodeling factor; hACF, human ACF; FACT, facilitates chromatin transcription; CHRAC, chromatin accessibility complex.

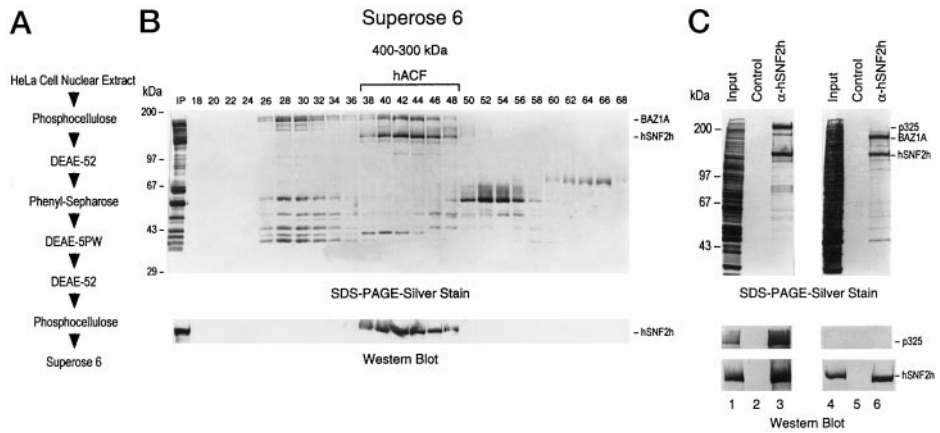

FIG. 1. **Purification of hACF.** *A*, chromatographic scheme used to purify hACF. *B*, *Top panel*: silver staining of a SDS-polyacrylamide gel containing an aliquot of the fractions derived from the last step of purification (Superose 6). *Bottom panel*, Western blot with hSNF2h antibody using Superose 6 fractions. *C*, *top panel*, silver staining of a SDS-polyacrylamide gel containing the immunoprecipitates of hSNF2h complexes. *Lane 1*, input for RSF immunoprecipitation; *lane 2*, RSF immunoprecipitation control; *lane 3*, immunoprecipitation of RSF with hSNF2h monoclonal antibody; *lane 4*, input for hACF immunoprecipitation; *lane 5*, hACF immunoprecipitation control; *lane 6*, immunoprecipitation of hACF with hSNF2h monoclonal antibody. *Bottom panel*, Western blot analysis of immunoprecipitation experiments with hSNF2h and RSF-p325 monoclonal antibodies.

remodeling and spacing activities. Like RSF, in combination with FACT, this complex is also capable of supporting transcription from chromatin templates in a defined system.

MATERIALS AND METHODS

Purification of hACF—Approximately 7.0 grams of HeLa nuclear extract were applied to 500 ml of phosphocellulose (Sigma) resin equilibrated in BC100 as described in Ref. 23. The proteins were step-eluted with 0.3 M, 0.5 M, and 1.0 M KCl washes. The hSNF2h polypeptide eluted in the 1.0 M fraction with ~10% of the total protein. This fraction was dialyzed against BC100 and then loaded on a 150-ml DEAE-52 (Whatman) column. The column was eluted with 0.3 M KCl and 0.5 M KCl washes. The hSNF2h protein eluted in the 0.3 M KCl fraction with half of the total protein. This fraction was dialyzed against buffer C containing 1.0 M $(NH_4)_2SO_4$, loaded onto a 55-ml phenyl-Sepharose (Amersham Pharmacia Biotech) fast protein liquid chromatography column and eluted with a linear gradient from 1.0 to 0 M $(NH_4)_2SO_4$. The hACF complex eluted between 0.3 and 0.2 M $(NH_4)_2SO_4$. The hSNF2h-containing fractions were pooled and dialyzed against buffer C containing 0.08 M $(NH_4)_2SO_4$, applied to a 38-ml DEAE-5PW high-performance liquid chromatography column (TosoHaas), and eluted with a linear gradient from 0.08 to 0.4 M $(NH_4)_2SO_4$. The hSNF2h protein eluted between 0.12 and 0.2 M $(NH_4)_2SO_4$. These fractions were pooled and dialyzed against buffer C containing 0.1 M $(NH_4)_2SO_4$ and was loaded onto a 1-ml DEAE-52 column equilibrated in the same buffer. The hSNF2h protein did not bind to the column; it was recovered in the column flow-through, while half of the total protein bound to the column. The DEAE-52 flow-through fraction was loaded directly onto a 1-ml phosphocellulose column and eluted with buffer C containing 1.0 M KCl. This fraction was then directly loaded onto a Superose 6 (Amersham Pharmacia Biotech) gel filtration column previously equilibrated in buffer C containing 0.5 M KCl. The hACF complex eluted with an apparent molecular mass of 300–400 kDa.

Immunoprecipitations—Immunoprecipitations were performed as described in Ref. 24. Monoclonal antibodies used for hSNF2h and RSF p325 were raised by Bios-Chile I.G.S.A. against the purified RSF complex selecting for hybridomas that produced antibodies against the separate subunits. The immunoprecipitations were washed with BC500 buffer C containing 0.5 M KCl and 0.05% Nonidet P-40. The input used for the RSF immunoprecipitation was derived from the third step of RSF purification as described in Ref. 11. The input used for hACF immunoprecipitation was from the phenyl-Sepharose.

Reconstituted Chromatin Assembly Assay—Recombinant (6×) histidine-tagged *Drosophila* NAP-1 was expressed from baculovirus in SF9 cells. The protein was purified by standard nickel affinity purification. Reconstituted chromatin assembly assays were performed by incubating 2 μg of plasmid DNA (pG5MLP), 1.9 μg of purified HeLa core histones, 150 μg of bovine serum albumin, 3 mM ATP, 30 mM phosphocreatine, 0.2 μg of phosphocreatine kinase, 5 mM $MgCl_2$, 50 mM KCl, 10 mM HEPES (pH 7.6), 0.2 mM EDTA, and 5% glycerol (v/v) and 100 ng of purified hACF at 30 °C for 5–6 h. After assembly the samples were split and digested with two different concentrations of micrococcal nuclease, deproteinated, run on a 1.2% agarose Tris-glycine gel, Southern blotted with a probe with sequence corresponding to the β-galactosidase gene on the plasmid.

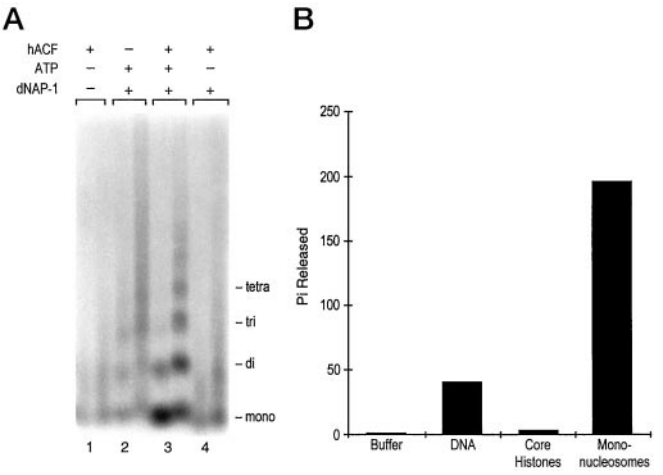

FIG. 2. *A*, reconstituted nucleosome assembly assay with dNAP-1 and purified hACF. *B*, ATPase assay with hACF. Reactions contained DNA, core histones, or mononucleosomes, as indicated on the *bottom* of the panel. Products of the reaction were separated by TLC, and the amount of $^{32}P_i$ released was quantitated and expressed as fold released over buffer.

Chromatin Assembly for Remodeling and Transcription Assays—Chromatin was assembled with *Drosophila* S-190 extract as described in Ref. 25 with 3 μg of plasmid DNA and 2 μg of HeLa-purified histones in a 400-μl volume. The plasmid DNA contains five GAL4 sites upstream of the adenovirus major late promoter followed by a 390-nucleotide G-less cassette. Chromatin was Sarkosyl-treated (0.05%) and purified away from the assembly extract as described in Ref. 23.

Nucleosome-remodeling Assay—The nucleosome-remodeling assay used was adapted from Tsukiyama *et al.* (11, 26). This assay uses periodically spaced nucleosomal templates assembled in *Drosophila* S190 extract, which have been Sarkosyl-treated and purified by gel filtration chromatography. The templates contain five GAL4-binding sites upstream of the adenovirus major late promoter. After incubating the chromatin templates with various combinations of ATP, GAL4-VP16, hACF, or RSF, the chromatin was digested with two different concentrations of micrococcal nuclease. The products were then analyzed by Southern blotting with probes within the vicinity of the promoter (promoter probe), stripped, and reprobed with a probe corresponding to a region 1000 base pairs away from the promoter (distal probe). Nucleosome remodeling is observed by a loss of periodic nucleo-

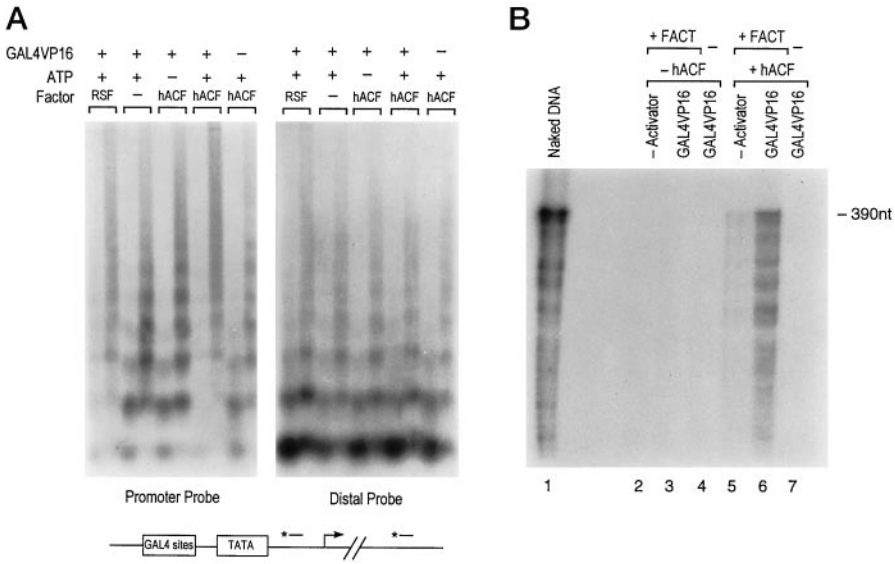

FIG. 3. *A*, purified hACF was assayed for nucleosome-remodeling activity. Purified RSF was used as a control. Additions were as indicated on the *top* of the panels. *Left panel*, shows a Southern blot hybridized with a probe corresponding to sequences within the promoter. *Right panel*, shows the same blot, which has been stripped and rehybridized with a probe corresponding to sequences 1000 base pairs away from the promoter. *B*, reconstituted transcription from chromatin templates facilitated by FACT and hACF. Productive full-length transcription is measured by the production of a 390-nucleotide transcript. *Lanes 2–7*, reconstituted transcription on chromatin templates. FACT, hACF, and GAL4-VP16 were added as indicated in the figure. *Lane 1* is full-length transcription reconstituted on naked DNA.

some spacing.

Chromatin Transcription Assay—The reconstituted chromatin transcription assay used was performed as described in (11), except the TFIID used was immunopurified with an anti-TBP antibody as described in Ref. 27.

ATPase Assay—The ATPase assay was performed as described in Ref. 28.

RESULTS

Isolation and Identification of the hACF Complex—To isolate different hSNF2h complexes we fractionated HeLa cell nuclei. Using antibodies to both subunits of RSF, we were able to find a fraction that contained hSNF2h, but was devoid of p325. We therefore set out to isolate a hSNF2h-containing complex from this fraction. Proteins were fractionated using a series of conventional chromatography columns as depicted in Fig. 1*A*. Each step was assayed by Western blot analysis for the hSNF2h protein. The final step of purification, a Superose 6 gel filtration column, is shown in Fig. 1*B*. The hSNF2h protein copurifies with a 190-kDa polypeptide, that together eluted with a native molecular mass of 300–400 kDa, suggesting that they form a heterodimer. We identified the 190-kDa polypeptide and confirmed the identity of hSNF2h by sequencing peptides generated by in-gel tryptic digestion with microcapillary high-performance liquid chromatography directly coupled to an ion trap mass spectrometer (11). Peptide sequences revealed that the 190-kDa polypeptide is BAZ1A, a newly identified protein with homology to *Drosophila* Acf1 (21).

To further analyze whether the hSNF2h protein is in two distinct complexes, we performed immunoprecipitation experiments with hSNF2h-specific antibodies (Fig. 1*C*). We used two different fractions for the immunoprecipitations, a crude but hSNF2h-enriched fraction that also contains RSF p325 or a crude but hSNF2h-enriched fraction devoid of RSF p325. When we used the crude fraction that contains RSF p325 (*lane 1*), we were able to coimmunoprecipitate both RSF subunits, p325, and hSNF2h (*lane 3*). When we used the crude fraction that is devoid of RSF (*lane 4*), we were able to coimmunoprecipitate both hACF subunits; the 190-kDa polypeptide (BAZ1A) and hSNF2h (*lane 6*). No polypeptides were observed in the control lanes where no antibody was used (*lanes 2* and *5*). The identity of RSF p325 polypeptide and the hSNF2h polypeptide were confirmed by Western blot (Fig. 1*C*, *bottom panel*).

The Nucleosome Spacing Activity of the hACF Complex—To determine whether hACF has nucleosome-spacing activity, we performed a chromatin assembly assay (Fig. 2*A*). Chromatin assembly was monitored by micrococcal nuclease digestion. As shown in *lane 3*, the presence of ATP, hACF, and dNAP-1, yielded nucleosome arrays with regular periodic spacing. In the absence of hACF or ATP, no appreciable arrays with periodic spacing were observed (*lanes 2* and *4*).

The ATPase Activities of the hACF Complex—To characterize the ATPase activity of hACF, we performed an ATPase assay in the presence of either sonicated calf thymus DNA (200–400 bp), mononucleosomes, or HeLa core histones. As shown in Fig. 2*B*, the hACF complex possesses DNA-dependent ATPase activity that is further stimulated by nucleosomes (6.8-fold when compared with DNA).

Nucleosome Remodeling Activity—We next tested the hACF complex for ATP-dependent nucleosome-remodeling activity. hACF exhibits ATP-dependent and activator-dependent nucleosome-remodeling (Fig. 3*A*). The remodeling is specific for the promoter region, because reprobing with a distal probe revealed regular periodic nucleosome spacing. Like RSF, hACF does not require an activation domain, because GAL4-(1–94), which lacks an activation domain, was also capable of directing nucleosome remodeling to the promoter region (data not shown).

Reconstituted Transcription from Chromatin Templates—Next, we tested hACF for its ability to allow the accessibility of transcription factors to chromatin templates using a reconstituted chromatin transcription assay. We have previously defined the requirement for a remodeling factor in our reconstituted chromatin transcription assays. The reconstituted transcription assay consists of the purified recombinant factors TFIIA, TFIIB, TFIIE, TFIIF, PC4, GAL4-VP16; the affinity-purified multisubunit factors TFIID, TFIIH, and RNA polymerase II; and highly purified FACT. The purified chromatin used for transcription assays was prepared in the same way as chromatin prepared for remodeling assays. As shown in Fig.

3*B*, robust transcription is facilitated in the presence of hACF and FACT from chromatin templates (*lane 6*). Transcription is greatly stimulated in the presence of an activator (*lane 5*). Transcription is severely compromised in the absence of either of these factors (*lanes 4* and *7*).

DISCUSSION

Our results indicate that the hSNF2h protein exists in at least two distinct complexes, the RSF complex and the hACF complex. Based on purification yields we estimate that there are approximately equal amounts of RSF and hACF in the cell. We believe that the abundance and redundancy of these enzymes is related to their function. In the nucleus, nucleosomes need to be fluid, so they can reorganize during and after factor binding or any DNA metabolism events. Since both RSF and hACF share the same ATPase subunit, the larger subunits, which make each complex unique, must specialize their function. The hACF complex is homologous to *Drosophila* ACF in both composition and function. It remains unknown whether there is an analogous complex to RSF in *Drosophila*. We had predicted in our previous publication that the *Drosophila* ACF complex would be able to substitute for RSF in our reconstituted chromatin transcription assays (11). Here we show that hACF is capable of fulfilling the requirement for a nucleosome-remodeling factor in the reconstituted chromatin transcription assays.

Another complex, purified from *Drosophila* is CHRAC (chromatin accessibility complex), which also contains the ISWI protein in addition to topoisomerase II and three other polypeptides (29). In the course of the purification of hACF we found that some of the hACF copurifies with topoisomerase IIβ. Using conventional chromatography, we were able to purify a complex containing these three polypeptides (hSNF2h, BAZ1A, and topoisomerase IIβ) to near-homogeneity. Moreover, this topoisomerase IIβ-containing complex eluted with an approximate mass of 600 kDa on a gel filtration column, which is 200 kDa larger than hACF, suggesting that they form a complex. Coelution of topoisomerase IIβ and hACF was also observed upon sucrose gradient sedimentation. However, we were unable to coimmunoprecipitate topoisomerase IIβ with hSNF2h-specific antibodies (data not shown). Whether the association between hACF and topoisomerase IIβ is functional remains to be elucidated. However, this complex is likely similar to CHRAC, which is believed to play a role during DNA replication (30).

Recently Bochar *et al*. (31) reported the isolation of a novel hSNF2h chromatin remodeling complex, which they termed WCRF. The authors indicated that WCRF is composed of a 180-kDa subunit in addition to hSNF2h. The 180-kDa subunit is encoded by a novel cDNA (WCRF180), which appears identical to BAZ1A. However, this group estimated the size of the complex to be 670 kDa by gel filtration chromatography. In light of our results demonstrating that hACF in association with topoisomerase IIβ, elutes from a gel filtration column with an apparent mass of 600 kDa, we speculate that the WCRF complex described by Bochar *et al.* (31) includes topoisomerase IIβ.

The hACF complex is the latest in the growing list of chromatin remodeling factors. Since there are many other sequences with homology to SNF2 in the DNA data bases, it is likely that this list will continue to grow.

Acknowledgments—We thank Dr. J. Kadonaga for the *Drosophila* NAP1 baculovirus expression system and Dr. Leroy Liu for topoisomerase IIβ antibodies.

REFERENCES

1. van Holde, K. E. (1989) *Chromatin* (Rich, A., ed), Springer-Verlag, Berlin, Germany
2. Luger, K., Mader, A. W., Richmond, R. K., Sargent, D. F., and Richmond, T. J. (1997) *Nature* **389,** 251–260
3. Noll, M., and Kornberg, R. D. (1977) *J. Mol. Biol.* **109,** 393–404
4. Spadafora, C., Bellard, M., Compton, J. L., and Chambon, P. (1976) *FEBS Lett.* **69,** 281–285
5. Wolffe, A. (1995) *Chromatin: Structure and Function*, Second Ed., London Academic Press, London
6. Tyler, J. K., and Kadonaga, J. T. (1999) *Cell* **99,** 443–446
7. Kingston, R. E., and Narlikar, G. J. (1999) *Genes Dev.* **13,** 2339–2352
8. Tsukiyama, T., Palmer, J., Landel, C. C., Shiloach, J., and Wu, C. (1999) *Genes Dev.* **13,** 686–697
9. Kornberg, R. D., and Lorch, Y. (1999) *Cell* **98,** 285–294
10. Gross, D. S., and Garrard, W. T. (1988) *Annu. Rev. Biochem.* **57,** 159–197
11. LeRoy, G., Orphanides, G., Lane, W. S., and Reinberg, D. (1998) *Science* **282,** 1900–1904
12. Ito, T., Bulger, M., Pazin, M. J., Kobayashi, R., and Kadonaga, J. T. (1997) *Cell* **90,** 145–155
13. Armstrong, J. A., Bieker, J. J., and Emerson, B. M. (1998) *Cell* **95,** 93–104
14. Mizuguchi, G., Tsukiyama, T., Wisniewski, J., and Wu, C. (1997) *Mol. Cell* **1,** 141–150
15. Yudkovsky, N., Logie, C., Hahn, S., and Peterson, C. L. (1999) *Genes Dev.* **13,** 2369–2374
16. Langst, G., Bonte, E. J., Corona, D. F., and Becker, P. B. (1999) *Cell* **97,** 843–852
17. Hamiche, A., Sandaltzopoulos, R., Gdula, D. A., and Wu, C. (1999) *Cell* **97,** 833–842
18. 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
19. Orphanides, G., Wu, W. H., Lane, W. S., Hampsey, M., and Reinberg, D. (1999) *Nature* **400,** 284–288
20. Lu, X., Meng, X., Morris, C. A., and Keating, M. T. (1998) *Genomics* **54,** 241–249
21. Jones, M. H., Hamana, N., Nezu, J., and Shimane, M. (2000) Genomics 63, 40–45
22. Ito, T., Levenstein, M. E., Fyodorov, D. V., Kutach, A. K., Kobayashi, R., and Kadonaga, J. T. (1999) *Genes Dev.* **13,** 1529–1539
23. Orphanides, G., LeRoy, G., Chang, C. H., Luse, D. S., and Reinberg, D. (1998) *Cell* **92,** 105–116
24. Zhang, Y., Iratni, R., Erdjument-Bromage, H., Tempst, P., and Reinberg, D. (1997) *Cell* **89,** 357–364
25. Bulger, M., and Kadonaga, J. T. (1994) *Methods Mol. Genet.* **5,** 241–262
26. Tsukiyama, T., and Wu, C. (1995) *Cell* **83,** 1011–1020
27. Wieczorek, E., Brand, M., Jacq, X., and Tora, L. (1998) *Nature* **393,** 187–191
28. Zhang, Y., LeRoy, G., Seelig, H. P., Lane, W. S., and Reinberg, D. (1998) *Cell* **95,** 279–289
29. Varga-Weisz, P. D., Wilm, M., Bonte, E., Dumas, K., Mann, M., and Becker, P. B. (1997) *Nature* **388,** 598–602
30. Varga-Weisz, P. D., Bonte, E., Becker, P. B., and Gross, C. (1998) *EMBO J.* **12,** 3428–3438
31. Bochar, D. A., Savarad, J., Wang, W., Lafleur, D. W., Moore, P., Cote, J., and Shiekhattar, R. (2000) *Proc. Natl. Acad. Sci. U. S. A.* **97,** 1038–1043