Biochemistry

 Volume 32, Number 40 October 12, 1993

Accelerated Publications

Unfolding of Nucleosome Cores Dramatically Changes the Distribution of Ultraviolet Photoproducts in DNA†

David W. Brown,‡ Louis J. Libertini,§ Christine Suquet,‡ Enoch W. Small,§ and Michael J. Smerdon*,‡

Washington State University, Pullman, Washington 99164-4660, and SIRTI Biotechnology Laboratory, Spokane, Washington 99207

Received July 13, 1993; Revised Manuscript Received August 16, 1993®

ABSTRACT: Nucleosome core particles undergo a conformational change at ionic strengths below 0.2 mM; the fluorescence anisotropy decay of bound ethidium indicates that under these conditions the particle adopts a highly extended structure. We have measured the distribution of UV-induced DNA damage (primarily cyclobutane–pyrimidine dimers) through a process termed photofootprinting. As the core particle is exposed to ionic strengths below 0.2 mM, the photofootprint pattern changes from that observed for native cores, with a characteristic 10.3 base repeat pattern presumably derived largely from the bending of DNA around the histone octamer, to a more evenly distributed pattern resembling that of free DNA. These results provide clear evidence that the DNA in the core particle at these very low ionic strengths, although still tightly bound to histones, is no longer bent to a significant degree.

The nucleosome core particle is a ubiquitous structure in eukaryotic chromatin, consisting of 146 bp[1] of DNA wrapped around an octamer of the core histones H2A, H2B, H3, and H4 (van Holde, 1989). Besides its obvious role in DNA packaging, this structure may also be used in the repression of certain genes by blocking the access of transcription factors to DNA sequences facing the histone surface (Hayes & Wolffe, 1992; Wolffe, 1992) or in the potentiation of transcription by creating a static loop which brings together widely separated sequences (Schild et al., 1993). The core particle has been shown to undergo a number of structural transitions *in vitro*, indicating that the particle is capable of adopting different metastable structures which could have the effect of rendering accessible these previously inaccessible regulatory sequences. In addition, some of these metastable structures may represent the transition states of nucleosomes during DNA processing events (i.e., transcription, replication, and repair).

Using a core particle preparation with a highly homogeneous DNA length (146 ± 2 bp) and such varied physical methods as tyrosine fluorescence anisotropy, circular dichroism spectroscopy, and sedimentation, we have consistently observed a conformational transition in the core particle just below 1 mM [reviewed in Brown et al. (1990)]. As long as the ionic strength is kept above 0.2 mM, this *low-salt* transition is reversible. If, however, the ionic strength of the solution is decreased below 0.2 mM, the transition becomes irreversible, with a much higher ionic strength being required to return the core particle to its initial state (Libertini & Small, 1987; Brown et al., 1991).

Using the fluorescence anisotropy decay of DNA-bound ethidium, Brown et al. (1991) showed that the irreversible form of the low-salt core particle is highly extended, having an axial ratio of at least 5:1. These results are compatible with two different models for the core particle structure at very low ionic strength (Brown, 1992; see Figure 4). The first model envisions a substantial *elongation* in the core particle in which the DNA is no longer highly curved but instead

† This work was supported by NIH Grants ES04106 and ES02614 (to M.J.S.) and GM25663 (to E.W.S.).

‡ Washington State University.

§ SIRTI Biotechnology Laboratory.

® Abstract published in *Advance ACS Abstracts*, September 15, 1993.

[1] Abbreviations: bp, base pair; CPD, cyclobutane–pyrimidine dimer; (6–4), pyrimidine (6–4) pyrimidone photoproduct; SDS–PAGE, sodium dodecyl sulfate–polyacrylamide gel electrophoresis.

0006-2960/93/0432-10527$04.00/0

becomes more linear. The second is a highly *flattened* structure in which the DNA remains curved, but to a much lesser degree, making only an ~0.9 complete turn about the histone core. Here the DNA essentially forms a loop with an outside radius of curvature of ~87 Å. This model is analogous to one proposed by Wu et al. (1979) on the basis of electric dichroism measurements, although our model must be much flatter, with a thickness of only ~35 Å, in order to obtain the 5:1 axial ratio consistent with the anisotropy decay data.

The very low salt transition of core particles occurs at such low ionic strengths (below 0.2 mM *total* ionic strength) that many analytical methods are unable to detect it. For example, hydroxyl radical footprinting, which would be expected to be able to detect changes in the helical repeat of the DNA (Hayes et al., 1990, 1991a,b; Bashkin et al., 1993), requires too high an ionic strength for the opening transition to occur. Similarly, neutron scattering measurements of the R_G of the DNA requires such concentrated solutions that again the total ionic strength is too high.

The UV photofootprinting technique described by Gale and Smerdon (Gale et al., 1987; Gale & Smerdon, 1988a) is particularly well suited for the study of the low-salt transition since the samples can be prepared at the very dilute, low-salt concentrations necessary for the opening transition to occur. The method involves measuring the distribution of UV-induced photoproducts in the core DNA [primarily CPDs and (6–4)s] by taking advantage of the fact that these lesions will block the 3′-exonuclease activity of T4 DNA polymerase (Doetsch et al., 1985). The resulting digestion products can then be separated on a DNA sequencing gel to visualize the photoproduct distribution pattern. We have previously shown that the UV photoproduct distribution in native core particles is highly modulated, with an average periodicity of 10.3 bases, reflective of structural features of the core particle such as histone–DNA interactions and/or bending of the DNA around the histone octamer (Gale et al., 1987; Gale & Smerdon, 1988a). This modulated pattern reflects only the distribution of CPDs, since the distribution of (6–4) photoproducts is almost completely random in core DNA (Gale & Smerdon, 1990).

Recently, Pehrson and Cohen (1992) used an artificial DNA sequence, consisting of two binding motifs for the λ repressor protein separated by five helical turns of diverse sequence DNA. When λ repressor is added, this DNA becomes bent to an outside radius of curvature of ~84 Å (Hochschild & Ptashne, 1986; see Discussion). These authors found that this DNA, in the absence of repressor protein, gave a random pattern of UV photoproducts while addition of the protein yielded an ~10-base periodicity, reminiscent of that seen in native core particles. Thus, DNA curvature seems to play a significant role in the modulation of photoproducts in nucleosome core DNA.

In order to determine if the photofootprint pattern characteristic of the compact core particle structure is altered when the core DNA is extended but still tightly bound by histones (Brown et al., 1990), we mapped the distribution of the UV-induced lesions in core particles at very low ionic strengths. Our results indicate that, at very low salt concentrations, the core particle DNA is no longer bent to a high degree and the modulated photofootprint pattern is abolished.

MATERIALS AND METHODS

Preparation of Nucleosome Core Particles. Nucleosome core particles were prepared from chicken erythrocytes as previously described (Libertini & Small, 1980; Libertini et al., 1988); concentrated stock solutions (A_{260} ~ 160) were prepared by extensive washings into 1 mM Tris, pH 8.0, using Centricon-30 miniconcentrators (Amicon). Free core particle DNA was prepared by proteinase K (Sigma Chemical Co.) digestion following standard procedures (Maniatis et al., 1982).

Irradiation of Samples. Core particle and free DNA solutions were prepared for irradiation by diluting stock solutions directly into solutions containing indicated amounts of added NaCl and incubating at room temperature for ~45 min before irradiation; the final A_{260} of the samples was 1.0. Samples (with a path length of ~2 mm) were irradiated at (predominantly) 254 nm using four low-pressure mercury germicidal lamps; the total UV dose was 500 J/m^2 (measured using a Spectronics DM-254N UV meter) to produce a damage yield of 1 CPD every one to two core particles (Gale & Smerdon, 1988b). Irradiated 146-base DNA was isolated from core particles by proteinase K digestion, followed by preparative denaturing polyacrylamide gel electrophoresis (Gale & Smerdon, 1988a) and electroelution of the 146-base band using an Amicon Centrilutor apparatus. This was done to remove small levels of overdigested DNA, resulting from micrococcal nuclease digestion during the core particle preparation, which increases the background in photoproduct mapping gels (Gale et al., 1987; Gale & Smerdon, 1988a).

Mapping of UV Photoproducts. The distribution of UV photoproducts was mapped as previously described (Gale & Smerdon, 1988a) with modifications as follows. One microgram of purified 146-base DNA was heat denatured and treated with 100 units of calf intestinal phosphatase (Boehringer Mannheim) to remove the 3′-terminal phosphate, labeled at the 5′ terminus with [γ-^{32}P]dATP (3000 Ci/mmol; New England Nuclear) and T4 polynucleotide kinase (U.S. Biochemicals), and then digested with T4 DNA polymerase (Boehringer Mannheim) in the absence of dNTP's. The samples were then ethanol precipitated and washed several times to remove short digestion products and excess salt. Samples were run on 6% denaturing acrylamide sequencing gels and autoradiographed (Gale & Smerdon, 1988a).

RESULTS

Characterization of Core Particles. The core particle preparation was characterized by polyacrylamide gel electrophoresis of both the histones and the DNA (Figure 1). In Figure 1A, decreasing amounts of core particles were separated by SDS–PAGE and stained with Coomassie Blue; it can be seen that only the four core histones are present, even at the higher loadings, with no apparent degradation. The core DNA was examined electrophoretically under both denaturing (Figure 1B) and native (Figure 1C) conditions. Figure 1B shows small amounts of overdigested DNA at high loadings; notable is the lack of significant amounts of underdigested DNA. Figure 1C shows free core DNA (lane S) electrophoresed under native conditions; comparison with molecular size markers (lane M) shows that the free DNA migrates as 146 ± 2 bp. The DNA from these preparations was then purified on denaturing polyacrylamide gels (Materials and Methods) for the photofootprinting analysis.

Photoproduct Distribution. Figure 2 shows an autoradiogram from a typical photofootprinting experiment. The modulated repeat pattern, characteristic of native core particles, can be clearly seen in lanes 1–5 (corresponding to concentrations from 10 to 0.2 mM added NaCl). The positions of the major ensemble peaks are indicated by the numbered arrows along the left side of the figure. Below 0.2 mM added salt, however, this modulated pattern begins to disappear as the ionic strength is lowered (lanes 6–9). The pattern in these

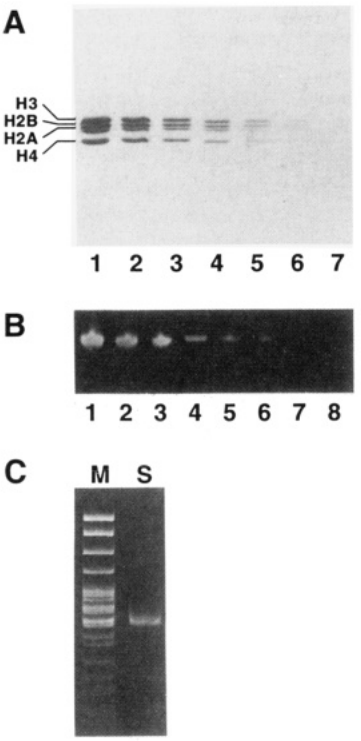

FIGURE 1: Characterization of core particle preparation. (A) Histones. Core particles at serial 2-fold dilutions (lane 1 = 4.3 μg of total protein) were treated with SDS, separated on an 18% [37.5:1 acrylamide–bis(acrylamide)] gel by SDS–PAGE (Ausubel et al., 1989), and stained with Coomassie Blue R-250; the positions of H3, H2B, H2A, and H4 are indicated. (B) Denatured DNA. Core particles at serial 2-fold dilutions (lane 1 = 4.0 μg of DNA) were electrophoresed on a 6% polyacrylamide gel [37.5:1 acrylamide–bis(acrylamide)] under denaturing conditions [7 M urea, 0.1% SDS (Ausubel et al., 1989)]; the DNA was visualized by staining with ethidium bromide. (C) Native DNA. Lane S: 0.5 μg of core DNA was electrophoresed on an 8% polyacrylamide gel [37.5:1 acrylamide–bis(acrylamide)] under native conditions [45 mM Tris, 45 mM boric acid, 1.25 mM EDTA (Ausubel et al., 1989)] and stained with ethidium bromide. Lane M: DNA size markers from a *Hpa*II digest of pBR322 DNA.

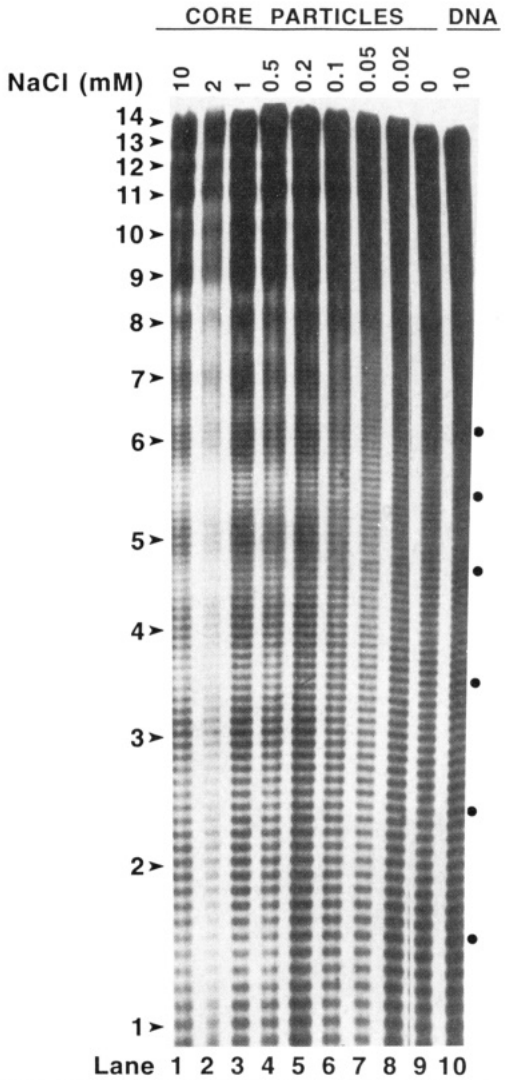

FIGURE 2: Autoradiogram of photofootprinting reactions. Lanes (numbered at bottom) are for core particles (1–9) or for free DNA (10) at the indicated concentrations of added NaCl. Major core particle ensemble peak positions are shown as numbered arrows along the left; minor free DNA ensemble peaks are indicated as dots along the right. The intensity difference for lane 2 is due to loading variation.

lanes more closely resembles that seen in lane 10, for core DNA irradiated as free DNA. Careful examination of lane 10 (and scan E in Figure 3) also reveals the presence of a slightly modulated pattern (indicated by the dots along the right-hand side of the autoradiogram), shifted by ~5 bases from that seen for the native core particle. This modulation most likely results from the asymmetric distribution of short A and T tracts in chicken core particle DNA (Drew & Travers, 1985; Satchwell et al., 1986; Gale & Smerdon, 1988a).

Figure 3 shows laser densitometer scans of selected lanes from Figure 2. Scan A is for core particles at 1 mM salt, at the onset of the low-salt transition. The characteristic modulated footprint pattern is clearly visible with a periodicity of ~10.3 bases (Gale et al., 1987; Gale & Smerdon, 1988a). Scan B is for cores at 0.2 mM salt, the point below which the low-salt transition becomes irreversible (Libertini & Small, 1987; Brown et al., 1991). The pattern observed is very similar to that seen in scan A (and also for the other higher salt concentrations tested; data not shown) and shows that the reversible low-salt transition does not cause any substantial change in DNA bending or histone occupancy, in agreement with previous results (Brown et al., 1991). Scans C and D are for core particles at 0.02 and 0 mM added NaCl, respectively. The *total* ionic strength in this last sample is estimated to be between 10 and 50 μM.[2] These scans represent the irreversible low-salt form of the core particle. The characteristic modulated pattern seen in scans A and B has clearly disappeared, being replaced by a more evenly distributed pattern. Indeed, these scans seem to resemble a mixture of both the core particle pattern and that produced by free core DNA (scan E), where the peaks and valleys of the former somewhat even out the corresponding valleys and peaks of the latter.

DISCUSSION

A schematic representation of the core particle at various stages throughout the low-salt transition is shown in Figure 4. Drawing A represents the core particle in its native, compact conformation, found above 1 mM ionic strength. These core particles show a characteristic 10.3-base average modulation in the distribution of UV damage (see Figure 3, scan A), similar to that seen in native nucleosome cores, isolated chromatin, and intact cells (Gale et al., 1987; Gale & Smerdon, 1988a; Jensen & Smerdon, 1990). This modulated pattern presumably reflects the bending of DNA around the surface

[2] Estimated by conductivity measurements using concentrated core particle stock solutions (D. W. Brown, L. J. Libertini, and E. W. Small, unpublished observations).

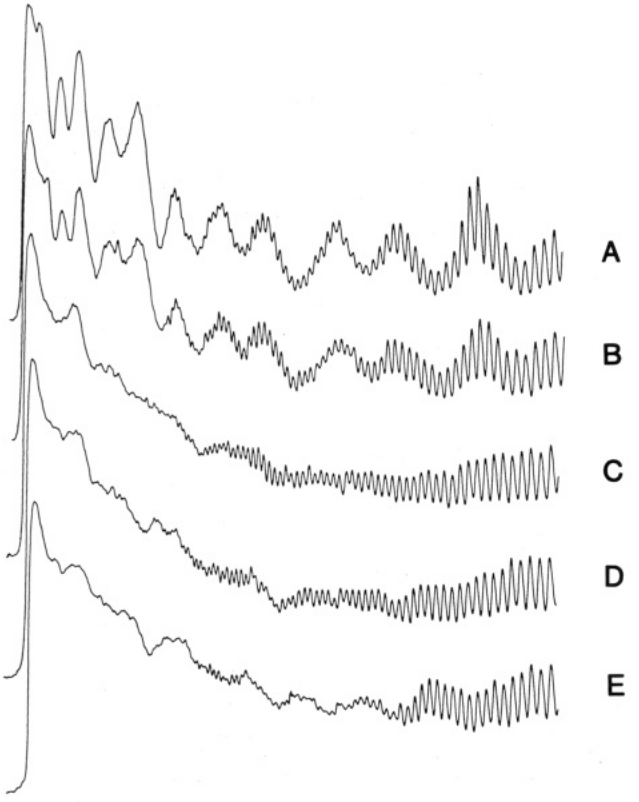

FIGURE 3: Densitometer scans of selected lanes from Figure 2. Lanes 3 (1 mM added NaCl), 5 (0.2 mM), 8 (0.02 mM), 9 (0 mM), and 10 (free DNA) were scanned with an LKB Ultroscan XL laser densitometer. The data were then smoothed before display by performing a three-bond running average over the entire data set (Hayes & Wolffe, 1990).

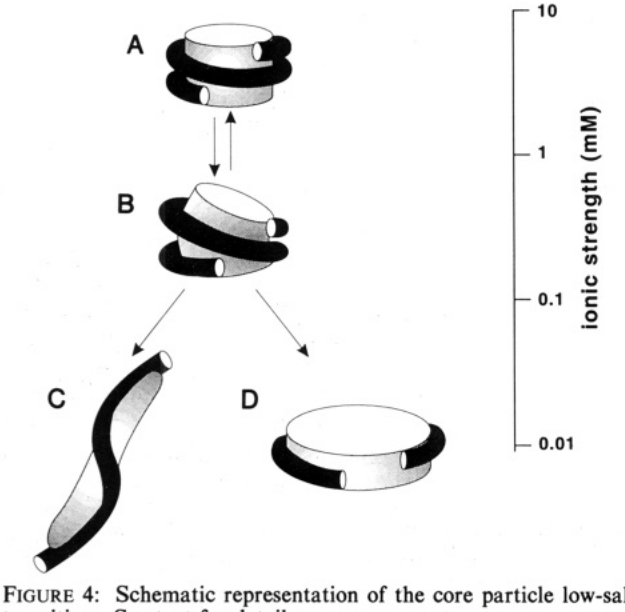

FIGURE 4: Schematic representation of the core particle low-salt transition. See text for details.

of the histone octamer (Gale et al., 1987; Pehrson & Cohen, 1992). Drawing B represents the reversible low-salt transition form of the core particle, slightly larger than in drawing A but still an essentially compact core. Histone H2B–H4 interactions are broken during this transition, but overall the pattern of histone–DNA interactions has remained largely intact (Martinson et al., 1979; Brown et al., 1991). These particles also show the modulated photofootprint pattern (Figure 3, scan B), suggesting that the DNA is still significantly bent around the protein core.

Drawing C represents the *elongated* model for the irreversible low-salt form of the core particle, adopted at ionic strengths below 0.2 mM. Increasing electrostatic repulsion between the adjacent, highly negatively charged DNA gyres presumably overcomes the H2A– and H3–DNA cross-binding interactions which are postulated to hold the core particle in its compact shape, allowing the superhelical stress stored in the core particle DNA to relax; the structure thus becomes highly elongated (Brown et al., 1991). It is also possible that additional histone–histone rearrangements are occurring during this irreversible unfolding of the core particle.

Drawing D represents the *flattened* model for the extended form of the core particle. Again, increasing electrostatic repulsion has caused the core particle to unfold, relieving superhelical stress in the DNA. Recent work by Pehrson and Cohen (1992) suggests that the curvature of DNA plays a major role in the development of the modulated UV photofootprint pattern. Their system was modified from that originally described by Hochschild and Ptashne (1986), who found that separation of the binding sites for λ repressor by an integral number of helical turns of DNA would still lead to cooperative binding by the repressor protein. They thus inferred that the DNA was being held in a loop by the tetrameric repressor. This DNA, based on the estimated height of the repressor protein, has an outside radius of curvature of ~84 Å (~60 Å for the protein plus ~24 Å for the diameter of the DNA). This value of ~84 Å is nearly identical to the ~87-Å radius predicted for the 5:1 flattened model of the core particle. Therefore, it would be expected that if the core particle adopted this highly flattened structure at very low ionic strength, one would still obtain a modulated UV footprint pattern. The results presented here, that this pattern is *not* seen when the core particle unfolds at very low ionic strength, argue strongly that the unfolded structure is highly elongated, as shown in drawing C.

While the low-salt transition occurs at ionic strengths which are clearly well below physiological levels, these results complement previous studies and demonstrate that the core particle is capable of adopting a highly elongated structure. It has been shown that removal of the unstructured N-terminal ends of the core histones, either by acetylation to disrupt their interaction with core DNA (Libertini et al., 1988) or by their complete removal via treatment with trypsin (Grigoryev & Krashennenikov, 1982), shifts the onset of the low-salt transition to much higher ionic strength. We have also found that the trypsinized core particle adopts the extended structure described here at about 5-fold higher ionic strength (Brown and Small, unpublished observations). One could imagine that some combination of internal histone modification and external factors could induce nucleosomes *in vivo* to adopt such an unfolded structure, one in which the DNA is now rendered much more accessible for processes such as transcription and repair.

REFERENCES

Ausubel, F. M., Brent, R., Kingston, R. E., Moore, D. D., Seidman, J. G., Smith, J. A., & Struhl, K. (1989) *Current Protocols in Molecular Biology*, Vol. 1, Wiley-Interscience, New York.

Bashkin, J., Hayes, J. J., Tullius, T. D., & Wolffe, A. P. (1993) *Biochemistry 32*, 1895–1898.

Brown, D. W. (1992) Conformational Transitions of Nucleosome Core Particles Monitored with Time-Resolved Fluorescence Spectroscopy, Ph.D. Thesis, Oregon State University, Corvallis, OR.

Brown, D. W., Libertini, L. J., & Small, E. W. (1990) *SPIE Proc. 1204*, 380–391.

Brown, D. W., Libertini, L. J., & Small, E. W. (1991) *Biochemistry 30*, 5293–5303.
Doetsch, P. W., Chan, G. L., & Haseltine, W. A. (1985) *Nucleic Acids Res. 13*, 3285–3304.
Drew, H. R., & Travers, A. A. (1985) *J. Mol. Biol. 186*, 773–790.
Gale, J. M., & Smerdon, M. J. (1988a) *J. Mol. Biol. 204*, 949–958.
Gale, J. M., & Smerdon, M. J. (1988b) *Biochemistry 27*, 7197–7205.
Gale, J. M., & Smerdon, M. J. (1990) *Photochem. Photobiol. 51*, 411–417.
Gale, J. M., Nissen, K. A., & Smerdon, M. J. (1987) *Proc. Natl. Acad. Sci. U.S.A. 84*, 6644–6648.
Grigoryev, S. A., & Krasheninnikov, I. A. (1982) *Eur. J. Biochem. 129*, 119–125.
Hayes, J. J., & Wolffe, A. P. (1992) *BioEssays 14*, 597–603.
Hayes, J. J., Tullius, T. D., & Wolffe, A. P. (1990) *Proc. Natl. Acad. Sci. U.S.A. 87*, 7405–7409.
Hayes, J. J., Bashkin, J., Tullius, T. D., & Wolffe, A. P. (1991a) *Biochemistry 30*, 8434–8440.
Hayes, J. J., Clark, D. J., & Wolffe, A. P. (1991b) *Proc. Natl. Acad. Sci. U.S.A. 88*, 6829–6833.
Hochschild, A., & Ptashne, M. (1986) *Cell 44*, 681–687.
Jensen, K. A., & Smerdon, M. J. (1990) *Biochemistry 29*, 4773–4782.
Libertini, L. J., & Small, E. W. (1980) *Nucleic Acids Res. 8*, 3517–3534.
Libertini, L. J., & Small, E. W. (1987) *Nucleic Acids Res. 15*, 6655–6664.
Libertini, L. J., Ausió, J., van Holde, K. E., & Small, E. W. (1988) *Biophys. J. 53*, 477–487.
Maniatis, T., Fritsch, E. F., & Sambrook, J. (1982) *Molecular Cloning, A Laboratory Manual*, Cold Spring Harbor Laboratory, Cold Spring Harbor, NY.
Martinson, H. G., True, R. J., & Burch, J. B. E. (1979) *Biochemistry 18*, 1082–1089.
Pehrson, J. R., & Cohen, L. H. (1992) *Nucleic Acids Res. 20*, 1321–1324.
Satchwell, S. C., Drew, H. R., & Travers, A. A. (1986) *J. Mol. Biol. 191*, 659–675.
Schild, C., Claret, F.-X., Wahli, W., & Wolffe, A. P. (1993) *EMBO J. 12*, 423–433.
van Holde, K. E. (1989) *Chromatin*, Springer-Verlag, New York.
Wolffe, A. P. (1992) *FASEB J. 6*, 3354–3361.
Wu, H.-M., Dattagupta, N., Hogan, M., & Crothers, D. M. (1979) *Biochemistry 18*, 3960–3965.