Histone acetylation: influence on transcription, nucleosome mobility and positioning, and linker histone-dependent transcriptional repression

Kiyoe Ura¹, Hitoshi Kurumizaka,
Stefan Dimitrov², Geneviève Almouzni³ and
Alan P.Wolffe⁴

Laboratory of Molecular Embryology, National Institute of Child Health and Human Development, NIH, Building 18T, Room 106, Bethesda, MD 20892-5431, USA

¹Present address: Department of Virology, Kurume University School of Medicine, 67 Ashahimachi, Kurume, Fukuoka 830, Japan

²Present address: Laboratoire d'Etudes de la Différentiation et de l'Adhrence cellulaire, UMR 5538, CNRS, Institut Albert Bonniot, Domaine de la Merci, 38706 La Tronche Cedex, France

³Present address: Institut Curie, Section de Biologie, 26 rue d'Ulm, 75231 Paris Cedex, France

⁴Corresponding author
e-mail: awlme@helix.nih.gov

We demonstrate using a dinucleosome template that acetylation of the core histones enhances transcription by RNA polymerase III. This effect is not dependent on an increased mobility of the core histone octamer with respect to DNA sequence. When linker histone is subsequently bound, we find both a reduction in nucleosome mobility and a repression of transcription. These effects of linker histone binding are independent of core histone acetylation, indicating that core histone acetylation does not prevent linker histone binding and the concomitant transcriptional repression. These studies are complemented by the use of a *Xenopus* egg extract competent both for chromatin assembly on replicating DNA and for RNA polymerase III transcription. Incorporation of acetylated histones and lack of linker histones together facilitate transcription by >10-fold in this system; however, they have little independent effect on transcription. Thus core histone acetylation significantly facilitates transcription, but this effect is inhibited by the assembly of linker histones into chromatin.

Keywords: acetylation/histones/nucleosome mobility/
nucleosome positioning/transcriptional regulation

Introduction

Histones and the chromatin structures they assemble have an active role in the transcription process (Felsenfeld, 1992; Grunstein *et al.*, 1992; Wolffe, 1995). Nucleosomes have been shown *in vitro* to both repress (Lorch *et al.*, 1987) and activate transcription (Schild *et al.*, 1993). *In vivo* experiments also indicate that chromatin components have a dual functional role in both activating and repressing gene activity (Kayne *et al.*, 1988; Durrin *et al.*, 1991; Fisher-Adams and Grunstein, 1995; Shen and Gorovsky, 1996). The exact role of chromatin structure in regulating any particular promoter in a natural context remains to be completely defined (Svaren and Horz, 1995, 1996).

In vitro experiments with model chromatin templates have proven useful in defining how the core histones and linker histones might restrict *trans*-acting factor access to nucleosomal DNA (Hayes and Wolffe, 1992a; Owen-Hughes and Workman, 1994). Certain *trans*-acting factors such as the glucocorticoid and thyroid hormone receptors bind efficiently to nucleosomal DNA (Perlmann and Wrang, 1988; Truss *et al.*, 1995; Wong *et al.*, 1995); others such as TFIID, Gal4 or TBP do not (Hayes and Wolffe, 1992a,b; Imbalzano *et al.*, 1994; Vettese-Dadey *et al.*, 1994; Godde *et al.*, 1995). Transcription factor association with nucleosomal DNA can be facilitated by removal of histone H1 (Juan *et al.*, 1994; Ura *et al.*, 1995), removal of core histones H2A/H2B (Hayes and Wolffe, 1992b; Chen *et al.*, 1994), proteolysis of the N-termini of the core histones (Lee *et al.*, 1993; Vettese-Dadey *et al.*, 1994; Godde *et al.*, 1995) or acetylation of the N-termini of the core histones (Lee *et al.*, 1993; Vettese-Dadey *et al.*, 1996). Of these various modifications to nucleosomal structure, acetylation is currently of most interest since the identification of transcription factors that can act as histone acetyltransferases (Brownell *et al.*, 1996; Mizzen *et al.*, 1996; Ogryzko *et al.*, 1996; Yang *et al.*, 1996).

High levels of histone acetylation are correlated with gene activity (Hebbes *et al.*, 1988, 1992, 1994; Tazi and Bird, 1990; Bartsch *et al.*, 1996) and reduced levels with gene silencing (Braunstein *et al.*, 1993). The patterns of acetylation on individual histones are complex (Turner and O'Neill, 1995), and exceptions exist to the general association of histone acetylation with transcription (O'Neill and Turner, 1995). Nevertheless, the fact that transcription factors can direct acetylation of the histones suggests that modification of the nucleosome may have a causal role in facilitating transcription. Newly synthesized histones H3 and H4 are acetylated (Ruiz-Carrillo *et al.*, 1975) and deacetylated shortly after their incorporation into the nascent chromatin assembled immediately following DNA replication (Jackson *et al.*, 1976). Newly synthesized H4 is invariably diacetylated at lysines 5 and 12 (Sobel *et al.*, 1995). It is in this form that H4 associates with chromatin assembly factor 1 (CAF1) (Verreault *et al.*, 1996). It has been proposed that the incorporation of acetylated histones into nascent chromatin might facilitate transcription factor access to DNA immediately following replication (Lee *et al.*, 1993; Verreault *et al.*, 1996).

We have approached the role of histone acetylation in the transcription of chromatin using a model dinucleosomal template reconstituted using purified histones (Ura *et al.*, 1995, 1996). This model template enables the characterization of both chromatin structure and transcriptional activity. In earlier work, we found that physiologically spaced histone octamers are mobile with respect to DNA sequence and repress transcription to 15–30% of the level of naked DNA (Ura *et al.*, 1995). Addition of histone H1 at a level

of one molecule per nucleosome restricts nucleosome mobility and further represses transcription (Ura *et al.*, 1995). Here we report that acetylation of the core histones significantly relieves transcriptional repression without influencing the mobility of histone octamers. Histone acetylation does not influence the repression of transcription by histone H1. These results are extended using a distinct experimental approach in which the acetylated histones are assembled into chromatin in a replication-coupled *in vitro* system (Almouzni and Méchali, 1988a,b; Almouzni *et al.*, 1991). We also find in this system that histone acetylation and linker histone deficiency significantly augment transcription.

Results

Histone acetylation facilitates transcription of dinucleosomal templates containing only histone octamers

We reconstituted a 424 bp template containing two *Xenopus* somatic 5S RNA genes with histone octamers (Ura *et al.*, 1995). We made use of the strong nucleosome positioning signals in the 5S RNA gene to separate nucleosomes (Simpson, 1991) and the capacity to have very efficient *in vitro* transcription of these genes as short linear DNA fragments *in vitro* (Wolffe *et al.*, 1986). Histone octamers were reconstituted onto radiolabeled DNA fragments by exchange from HeLa cell chromatin enriched in acetylated core histones or from chromatin with minimal levels of histone acetylation (see Figure 1C, Ura *et al.*, 1994). The reconstituted chromatin was fractionated on a sucrose gradient and each fraction was analyzed by nucleoprotein gel electrophoresis. Free DNA was resolved from mono-, di- and trinucleosomal complexes (data not shown, Ura *et al.*, 1995). Transcription of naked DNA, mono- and dinucleosome templates reconstituted with histones having minimal levels of acetylation, in extracts of *Xenopus* oocyte nuclei under efficient conditions (Birkenmeier *et al.*, 1978; Wolffe *et al.*, 1986), reveals a progressive reduction in transcriptional activity concomitant with the increase in the number of histone octamers bound (Figure 1A). Our results assay three predominant types of transcripts (Wolffe *et al.*, 1986; Hansen and Wolffe, 1992, data not shown): there are transcripts that initiate at the start site of the 5S rRNA gene and terminate at the end of the gene (experimental 5S rRNA transcript), other transcripts initiate at the start site of the 5S rRNA gene and fail to terminate at the end of the gene continuing to the end of the DNA fragment (read-through transcripts), and transcripts that initiate at the end of the DNA fragment and continue the entire length of the fragment (end-initiated transcript). These transcripts provide information concerning both the access of the transcriptional machinery to the regulatory elements of the 5S rRNA gene (experimental 5S rRNA transcript) and the processivity of RNA polymerase III through chromatin templates (read-through transcripts and end-initiated transcripts). In all of our experiments, we find that all of the transcripts change in abundance to very similar extents; this is indicative of a general change in the access of the transcriptional machinery to DNA in response to changes in chromatin composition. We also include a naked internal control plasmid template which

encodes a maxi 5S rRNA gene in our transcription reactions (maxi-gene internal control, Bogenhagen *et al.*, 1982); this template demonstrates that the repressive effects of chromatin are specific to the nucleosomal template. Finally, we can assay the recovery of nucleic acid from the transcriptional reaction from the abundance of the radiolabeled DNA (DNA) that serves as the template for transcription. Quantitation of the transcriptional repression due to reconstitution of dinucleosomes with minimally acetylated core histones (using a Phosphorimager, data not shown) indicates an 85% reduction in transcriptional activity (Figure 1A, compare lanes 1 and 3).

Our next experiments examined the differences between the transcriptional activity of mononucleosomes and dinucleosomes reconstituted with core histones having different levels of acetylation (Figure 1B). We compared transcriptional activity in reactions to which no histone deacetylase inhibitor was added (Figure 1B, lanes 1–4), or to which the deacetylase inhibitors sodium butyrate (lanes 5–8) or trichostatin A (lanes 11–12, Yoshida *et al.*, 1995) were added. In each instance the results were very similar. Histone acetylation was without significant consequence for transcription of mononucleosomal templates; however, high levels of histone acetylation facilitate the transcription of dinucleosomal templates 5- to 8-fold (Figure 1B brackets, compare lanes 3 and 4, lanes 7 and 8, and lanes 11 and 12). Thus acetylation of core histones facilitates the transcription of dinucleosomal templates containing only histone octamers.

The histones on the dinucleosomal templates purified on the sucrose gradients are shown in Figure 1C. The octamers enriched in acetylated core histones contain histone H4 with a distribution of acetylated isoforms containing predominantly two, three and four acetylated lysines (lane 1). Use of antibodies against acetylated lysine 16 (H4. Ac 16, Turner *et al.*, 1992) indicates reactivity with all these H4 isoforms, as anticipated from earlier work (S.Dimitrov, data not shown; Turner and O'Neill, 1995). In contrast, the dinucleosomal templates assembled using histones containing minimal levels of histone acetylation contained core histones in which no significant levels of acetylated H4 isoforms were present (Figure 1C, lane 2) and that did not react with antibodies against acetylated Lys16 (S.Dimitrov, data not shown). We examined whether histone acetylation was maintained through the time course of the experiment (Figure 1D). The relative abundance of acetylated isoforms of histone H4 is maintained during the transcription reaction in the presence of butyrate (compare lanes 1 and 2) or trichostatin A (compare lanes 1 and 3). In the absence of histone deacetylase inhibitors there is a partial loss of acetylated isoforms which decline from an average of three to that of two acetylated lysines per histone H4 (data not shown). The core histones also remain intact during the transcription reaction (Figure 1D, lanes 4 and 5). Having established that the histones and their acetylation state were maintained under the conditions of transcription, we next wished to determine what structural properties of dinucleosomal template might change in response to acetylation and contribute to facilitating transcription.

The influence of histone acetylation on nucleosome mobility and position

Nucleosome mobility has been suggested to have a major role in allowing access of the transcriptional machinery

Fig. 1. The influence of histone acetylation on transcription of dinucleosome templates reconstituted with histone octamers. **(A)** Repression of transcription depends on the number of control histone octamers with minimal histone acetylation reconstituted on the X5S 197-2 DNA template. Radiolabeled DNA (DNA) template was reconstituted with histone octamers, and mono- and dinucleosome products were separated by sucrose gradient centrifugation. These complexes were then used as templates for transcription together with a naked plasmid template encoding a maxi 5S RNA gene as an internal control in an extract of *Xenopus* oocyte nuclei. Lane 1, naked DNA; lane 2, mononucleosome; lane 3, dinucleosome. Transcripts were analyzed by electrophoresis in a 6% denaturing polyacrylamide gel. The positions of 5S rRNA (Experimental 5S rRNA transcript), the maxi-gene 5S transcript (Maxi-gene internal control), transcripts initiated at the first 5S rRNA gene that read-through the first termination signal (Read-through transcript) and transcripts initiated at either end of the DNA fragment that read through the length of the DNA sequence (End-initiated transcript) are indicated. **(B)** Influence of histone acetylation on transcription of the X5S 197-2 DNA template reconstituted with a single (mono-) or two (di-) histone octamers. Radiolabeled DNA was reconstituted with octamers containing acetylated (+) or control histone with minimal acetylation (–). Mono- and dinucleosome products were separated by sucrose gradient centrifugation. These complexes were then used as templates for transcription together with a naked plasmid template encoding a maxi 5S RNA gene as an internal control, in an extract of *Xenopus* oocyte nuclei. These transcription reactions were carried out with no additions (lanes 1–4), or with addition of 10 mM sodium butyrate (lanes 5–8) or 10 μ M Trichostatin A (lanes 9–12). Transcripts are indicated as in (A). Transcripts derived from the dinucleosomal templates in the presence (+) or absence (–) of histone acetylation are indicated by the bracket. **(C)** Histones reconstituted onto the X5S 197-2 DNA templates. Dinucleosomes were reconstituted using hyperacetylated or hypoacetylated donor chromatin before separation on a sucrose gradient. The dinucleosome fractions were pooled and resolved on Triton acid–urea gels (Materials and methods). Acetylated dinucleosomes (lane 1) and control dinucleosomes (lane 2) are shown. The positions of histone H4 (H4) and various acetylated isoforms are indicated (0–4). **(D)** Stability of histone acetylation states during the transcription reaction. Dinucleosomes were reconstituted onto the X5S 197-2 DNA template using hyperacetylated donor chromatin before separation on a sucrose gradient. These dinucleosomes were used for transcription in the oocyte nuclear extract. Dinucleosomes incubated under the conditions described in (B) were resolved on a Triton acid–urea gel (lanes 1–3) or by SDS–18% PAGE (lanes 4–7) at the end of the 40 min reaction. Lane 1 shows the histones on the dinucleosomes before addition of oocyte nuclear extract, lane 2 after 40 min incubation in the presence of 10 mM sodium butyrate (+Butyr) and lane 3 after 40 min incubation in the presence of 10 μ M Trichostatin A (+Tricho). Lanes 4 and 5 are as in 2 and 3 except that histones were resolved on SDS–PAGE gels. Lanes 6 and 7 are BRL molecular weight standard and chicken erythrocyte histones as markers respectively.

to regulatory DNA (Meersseman *et al.*, 1992; Becker, 1994; Ura *et al.*, 1995; Varga-Weisz *et al.*, 1995). We examined the influence of histone acetylation on the mobility of histone octamers relative to DNA sequence. To assess nucleosome mobility, we made use of electrophoresis on non-denaturing polyacrylamide gels (Meersseman *et al.*, 1992; Pennings *et al.*, 1994). Resolution of nucleoprotein complexes on polyacrylamide gels is sensitive to the conformation of the histone–DNA complex (Meersseman *et al.*, 1992). Thus a single histone octamer associated with a 424 bp DNA fragment can be resolved into multiple complexes on a single dimension of electrophoresis dependent on the translational position of the histone octamer along the DNA fragment. Evidence for the mobility of a histone octamer comes from carrying

out a second dimension of electrophoresis (Meersseman *et al.*, 1992). If the octamer changes position during a 1 h incubation at 4 or 37°C prior to the second dimension, then this will be detected by the appearance of a nucleoprotein complex that migrates at a position away from a simple diagonal. Nucleosome mobility is temperature dependent: using a 424 bp DNA fragment reconstituted with histone octamers containing high levels of acetylation (Figure 2A) or minimal levels of acetylation (Figure 2B), more nucleoprotein complexes migrate off the diagonal at 37°C than at 4°C. Acetylated histone octamers do not show more mobility than control octamers. Two-dimensional analysis of dinucleosomal templates reconstituted with two histone octamers again reveals comparable levels of nucleosome mobility with acetylated (Figure 2C) and control octamers

Fig. 2. The influence of histone acetylation on nucleosome mobility. Mononucleosomes assembled using acetylated (A) or control histones with minimal acetylation (B) were resolved on a 4% polyacrylamide gel at 4°C before excision of the gel lane and incubation at 4 or 37°C for 1 h as indicated, followed by a second dimension of electrophoresis (Materials and methods). The directions of electrophoresis are indicated. (C and D) As in (A) and (B) except dinucleosomes were used.

Fig. 3. Micrococcal nuclease digestion of acetylated and control mononucleosomes and dinucleosomes. Mononucleosomes (lanes 1–4 and 6–9) and dinucleosomes (lanes 11–18) were reconstituted using acetylated (lanes 1–4 and 11–14) or control histones (lanes 6–9 and 15–18) before fractionation on a sucrose gradient. Purified material was digested with 0.075, 0.15, 0.3 and 0.6 U of micrococcal nuclease (5 min, 22°C) as indicated by the triangles above the lanes. Products of digestion were labeled with [γ - 32 P]ATP and analyzed by native polyacrylamide gel electrophoresis. Lanes 5 and 10 contain *MspI*-digested pBR322 size markers. The position of the 146 bp core particle size DNA fragment (Core) as a product of digestion is indicated.

(Figure 2D). We conclude that histone acetylation does not have a major influence on the mobility of histone octamers with respect to DNA sequence.

We next made use of an independent nuclease mapping methodology (Meersseman *et al.*, 1991) to investigate nucleosome mobility through the associated variation in nucleosome position. Micrococcal nuclease digestion of acetylated and control nucleosomal complexes results in the accumulation of core particle size DNA (core, 146 bp) as a kinetic intermediate in digestion (Figure 3).

Both acetylated (lanes 1–4) and control mononucleosome reconstitutes (lanes 6–9) show comparable digestion kinetics, as do acetylated (lanes 11–14) and control dinucleosomes (lanes 15–18). These digestions were followed by restriction endonuclease digestion of the core particle sized DNA. This allows the mapping of the boundaries of the histone octamer relative to the 5S rRNA gene sequences on the dinucleosome template. A single histone octamer adopts a preferred location with boundaries at –68 and +79 relative to the transcription start site (+1) of either 5S rRNA gene independent of acetylation status (Figure 4A, lanes 1–11, dots show the 5' and 3' boundaries of the histone octamer, Figure 4B). When two histone octamers are present, this preference is less apparent (Figure 4A, lanes 12–22). We find that the 5' histone octamer adopts at least five distinct translational positions independent of histone acetylation (Figure 4A, compare lanes 13–16 with 18–21, dots show the 5' boundaries of the octamer). These multiple translational positions indicate nucleosome mobility (Meersseman *et al.*, 1991, 1992). A unique restriction site (*Fnu*4HI, Figure 4B, marked by an asterisk) allows discrimination between the boundaries of DNA contact made by the 5' and 3' histone octamers (Figure 4A, lanes 6, 11, 16 and 21). The 3' histone octamer has fewer positions and, therefore, appears less mobile than the 5' octamer (Ura *et al.*, 1995). There is also a fraction of DNA that remains resistant to cleavage by *EcoRV* (Figure 4A, lanes 4, 9, 14 and 19). Cleavage by *Hgi*AI in addition to *EcoRV* (lanes 5, 10, 15 and 20) indicates that most of this core particle DNA is present within a population of nucleosomes that have the 5' end of the DNA fragment as their 5' boundary. The proportion of nucleosomes in this fraction is also unchanged by histone acetylation. Thus we conclude that alterations in nucleosome positioning or the stability with which nucleosomes maintain their position with respect to DNA sequence do not occur in response to acetylation of the core histones.

Linker histone binding restricts nucleosome mobility and represses transcription independently of core histone acetylation

Acetylation of the core histones correlates with a deficiency of linker histones in newly assembled chromatin (Perry

Fig. 4. Micrococcal nuclease mapping of core positions on reconstituted mono- and dinucleosomal complexes. DNA from the nucleosome core particles as described in Figure 3 was recovered from an acrylamide gel (Figure 3) and digested with restriction endonucleases. (A) Mapping of DNA fragments. Predominant products of *EcoRV* (E) digestion of core particle DNA were 129, 100, 46 and 17 bp fragments, labeled a, b, b' and a', respectively (lane 4). Further digestion with *HgiAI* (H) and *Fnu4HI* (F) allows the exact positions of the DNA fragments to be determined. For the dinucleosome substrates as shown in lanes 14 and 19, a ladder of bands is visible below the 129 bp band separated by 10–11 bp dots. These represent the multiple positions occupied by the histone octamer on the 5' 5S rRNA gene repeat. Cleavage with *EcoRV* plus *Fnu4HI* (lanes 6, 11, 16 and 21) allows resolution of the 5' boundaries of the 3' octamer and leads to the accumulation of a novel 23 bp fragment labeled c. The upper bands represent the boundaries of the octamers on the 3' 5S rRNA gene repeat and the novel fragment the DNA sequence (c) from the 5' *Fnu4HI* site to the *XbaI* site in the 5' 5S rRNA gene repeat. Size markers (lanes 2, 7, 12 and 17) and DNA fragments from a hydroxyl radical cleavage reaction (lanes 1 and 22), which provide a more accurate indication of fragment sizes intermediate to the size markers, are shown. (B) Location of restriction fragments shown in (A). Thick arrows are the 5S rRNA genes. Open boxes are the internal control regions which are the binding sites for TFIIIA.

Fig. 5. Binding of histone H1 to reconstituted acetylated and control dinucleosomes represses transcription. (A) Binding of H1 to reconstituted dinucleosomes. Reconstituted dinucleosomes containing acetylated (lanes 1–6) or control core histones (lanes 7–12) were mixed with free DNA before various amounts of histone H1 were added and analyzed by nucleoprotein agarose (0.7%) gel electrophoresis. Lanes 1–6, 100 ng (DNA content) of reconstituted acetylated dinucleosomes (= 0.6 pmol) were mixed with 0, 0.3, 0.6, 1.2, 2.4 and 4.8 pmol of H1 respectively. Lanes 7–12 are as for 1–6, except that control core histones with minimal acetylation were used. Dinucleosomes containing one (1) and (2) molecules of histone H1 per dinucleosome are indicated. Note that nucleosome mobility (Meersseman *et al.*, 1992) interferes with the resolution of the nucleoprotein complexes. (B) Binding of histone H1 represses transcription. The X5S 197-2 DNA template reconstituted with two histone octamers containing acetylation histones (lanes 2 and 3) or control histones with minimal acetylation (lanes 5 and 6) were separated on sucrose gradients. To 100 ng of reconstituted dinucleosome (0.6 pmol) was added 1.2 pmol of histone H1 (lanes 3 and 6) or H1 was not added (lanes 2 and 5) before 10 ng of the template was transcribed in the oocyte nuclear extract. As a control, 10 ng of naked X5S 197-2 DNA fragment was transcribed (lanes 1, 4 and 7). To all transcription reactions a naked plasmid template encoding a maxi 5S rRNA gene as an internal control was added. Transcripts and DNA are as described in Figure 1A.

and Annunziato, 1989, 1991). It has also been suggested that core histone acetylation interferes with the association of linker histones with mononucleosomes (Juan *et al.*, 1994). In contrast to these results, we have found that the chromatin of *Xenopus* embryonic nuclei in which core histones are maintained in a hyperacetylated state incorporate normal stoichiometries of histone H1 (Dimitrov *et al.*, 1993). Moreover, mononucleosomes assembled using acetylated core histones and the *Xenopus* 5S rRNA gene also incorporate histone H5 without impediment (Ura *et al.*, 1994). However, since dinucleosomes offer the opportunity to examine histone H1-dependent transcriptional repression (Ura *et al.*, 1995, 1996), and since histone H1 binds with different affinities to mononucleosome compared with dinucleosome substrates (Hayes *et al.*, 1996; Nightingale *et al.*, 1996a,b; Ura *et al.*, 1996), we next explored whether linker histones would stabilize acetylated histone octamers comparably to controls and whether transcription would be repressed with similar efficiency.

We find that histone H1 binds with equivalent efficiency to dinucleosomal templates reconstituted with acetylated (Figure 5A, lanes 1–6) or control histone octamers (Figure 5A, lanes 7–12). These nucleoprotein complexes also aggregate with equivalent efficiency (Figure 5A, lanes 6 and 12). Control experiments indicated that chromatinosome (166 bp) intermediates in micrococcal nuclease

digestion accumulated in the presence of histone H1 independently of core histone acetylation state (data not shown; Ura *et al.*, 1994). We next examined the consequences of incorporating histone H1 for transcription of the dinucleosomal templates.

In earlier work, we had established that the incorporation of histone H1 into a dinucleosomal template would restrict transcription of the 5S rRNA genes (Ura *et al.*, 1995). We repeated these experiments using dinucleosomes containing acetylated or control core histones, incorporating trichostatin into the reaction mixture to impede deacetylation of the core histones (see Figure 1). We find that histone H1 effectively represses transcription of dinucleosomal templates to comparable levels, independently of histone acetylation state (Figure 5B, compare lanes 2 and 3 with 5 and 6). The repression of transcription dependent on histone H1 has been attributed to the restriction in the mobility of histone octamers relative to the 5S rRNA gene (Ura *et al.*, 1995). We next examined the mobility of histone octamers on the dinucleosomal template reconstituted with acetylated or control core histones in the presence or absence of histone H1. Histone H1 restricted nucleosome mobility at 37°C independently of core histone acetylation (data not shown). We conclude that the correlation between the restriction in nucleosome mobility and transcriptional repression is maintained and that histone H1 retains the capacity to repress transcription from acetylated chromatin.

In earlier work, we and others had observed that histone acetylation had no influence on the rotational positioning of DNA on the surface of the histone octamer (Norton *et al.*, 1990; Bauer *et al.*, 1994). We had also found that incorporation of linker histones into mono- or dinucleosomal templates had no influence on the rotational positioning of DNA on the surface of the histone octamer(s) (Hayes *et al.*, 1993; Ura *et al.*, 1995). Since the emphasis of the experiments reported here is on constraints on the mobility of the histone octamer with respect to DNA sequence, we wished to establish whether the DNA might adopt different rotational positions in the dinucleosome dependent on histone acetylation and/or histone H1. We find that there is no change in the rotational positioning of DNA with respect to the histone surface dependent on either acetylation or linker histones, as detected by hydroxyl radical cleavage (data not shown).

Diacetylation of histone H4 and linker histone deficiency together facilitate the assembly of transcriptionally competent chromatin during replication

Our experiments using model dinucleosomal templates demonstrate that acetylation of the core histones can facilitate transcription in the absence of histone H1. These studies make use of an experimental strategy requiring the prior assembly of chromatin of defined composition (Figure 1C) before addition to a transcription extract enriched in transcription factors for RNA polymerase III and deficient in chromatin assembly itself (Birkenmeier *et al.*, 1978; Wolffe *et al.*, 1986, 1987). Thus the facilitation of transcription attributed to acetylation of the core histones occurs *after* assembly of the histone octamer is complete. This might reflect the consequences of the potentially targeted acetylation of the core histones conferred by

transcription factors (Brownell *et al.*, 1996; Mizzen *et al.*, 1996; Ogryzko *et al.*, 1996; Yang *et al.*, 1996). However, the consequences for transcription might be very different when acetylated core histones are incorporated into nascent chromatin following DNA replication (Ruiz-Carrillo *et al.*, 1975; Verreault *et al.*, 1996). To investigate this issue, we made use of a physiological chromatin assembly system. *Xenopus* egg extracts will assemble chromatin in a replication-coupled reaction during second strand synthesis on single-stranded DNA templates (Almouzni and Méchali, 1988a,b; Almouzni *et al.*, 1990a). This system has proven useful in determining the role of various core histones in directing transcriptional repression during the staged assembly of chromatin (Almouzni *et al.*, 1990b, 1991). Histone H4 is stored in a diacetylated form in *Xenopus* eggs (Figure 6A, panel 1, Shimamura and Worcel, 1988; Dimitrov *et al.*, 1994). In agreement with predictions (Sobel *et al.*, 1995), the use of antibodies specific for acetylated H4 isoforms indicates that lysines 5 and 12 are acetylated in this storage form (S. Dimitrov, data not shown; Turner and Fellows, 1989). Following incorporation into chromatin during embryogenesis, histone H4 is progressively deacetylated (Figure 6A, panels 3 and 4). This deacetylation can be inhibited with sodium butyrate (Figure 6A, panel 2). The *Xenopus* egg extract does not contain histone H1, but does contain an oocyte-specific linker histone variant, histone B4 (Dimitrov *et al.*, 1994; Nightingale *et al.*, 1996a). Immunoblotting (Figure 6B) indicates that maintenance of histone diacetylation does not influence the incorporation of histone B4 into chromatin. Thus we can explore the role of histone H4 diacetylation and linker histone B4 in the repression of 5S rRNA gene transcription.

We depleted histone B4 from *Xenopus* egg extract using established protocols (Figure 6C, Dasso *et al.*, 1994; Dimitrov *et al.*, 1994) and examined the assembly of nucleosomes in the presence or absence of sodium butyrate (Figure 6D). We find that nucleosomes are assembled with comparable efficiency (data not shown) and comparable resistance to micrococcal nuclease independently of sodium butyrate (Figure 6D, compare lanes 1–6 with 7–14). There is a slight increase in nuclease sensitivity following depletion of histone B4 (Figure 6D, compare lanes 1–3 and 7–10 with 4–6 and 11–14). Our next experiments examined the consequences for the transcription of two class III genes: satellite I DNA (Wolffe, 1989) and a 5S rRNA gene, that are assembled into chromatin under these various conditions on a replicating template. As a control, we made use of double-stranded DNA templates that are assembled into chromatin inefficiently and that remain transcriptionally competent under these reaction conditions (Almouzni *et al.*, 1990b, 1991). We find that the depletion of histone B4 from the extract leads to a modest 2-fold stimulation of transcription (Figure 6E, compare lanes 3 and 4), as does the addition of sodium butyrate (Figure 6E, compare lanes 3 and 5). However, when the extract is both depleted of histone B4 and maintained in the presence of sodium butyrate, there is a >10-fold augmentation of transcription (Figure 6E, compare lanes 3 and 6). Transcription levels become similar to those of a comparable mass of double-stranded DNA that is only inefficiently assembled into chromatin in this system (Figure 6E, compare lanes 1 and 6). Thus

Fig. 6. (A) Histone H4 acetylation states during minichromosome assembly. Silver-stained two-dimensional gels are shown (Materials and methods) of proteins isolated from minichromosomes assembled as described (Materials and methods). Panel 1: minichromosomes were fractionated on sucrose gradients and the proteins analyzed by two-dimensional gel electrophoresis. The positions of the core histones and the various acetylated forms of histone H4 are indicated. Panel 2: proteins assembled into minichromosomes after 60 min in extracts to which sodium butyrate has been added (+NaBu). Panel 3: proteins assembled into minichromosomes after 30 min in extracts to which sodium butyrate has not been added (–NaBu). Panel 4: protein assembled into minichromosomes after 60 min in extracts to which sodium butyrate has not been added (–NaBu). The positions of the four core histones are indicated, as are the positions of deacetylated (0), monoacetylated (1) and diacetylated (2) histone H4. (B) Minichromosomes assembled for 2 h with or without sodium butyrate were bound to nitrocellulose as indicated. The slot blots were then incubated with antibodies specific for histones B4 (B4), H2B (H2B) or pre-immune serum (control from rabbits subsequently injected with histone B4). (C) Slot-blot with antibodies against B4 of the nitrocellulose-bound proteins associated with minichromosomes that are assembled in egg extract mock-depleted (lane 1 Mock) or depleted for B4 (lane 2). The upper slot contains twice the protein of the lower. (D) Minichromosomes assembled in the egg extract for 60 min in the presence (Mock) or absence of B4 (B4 dep) with or without sodium butyrate were digested with micrococcal nuclease: 1 U, lanes 1, 4, 7 and 11; 5 U, lanes 2, 5, 8 and 12; 20 U, lanes 3, 6, 9 and 13; and 50 U, lanes 10 and 14. An autoradiogram is shown of purified DNA resolved on a 1.5% agarose gel. The size of marker DNA fragments (Gibco BRL, Gaithersburg, MD) is shown. (E) Transcription of minichromosomes assembled under the various conditions indicated in (D). Chromatin was assembled on replicating single-stranded DNA containing satellite I DNA and 5S rRNA gene for 1 h, and transcription assayed between 1 and 2 h. The positions of transcripts from satellite I DNA (Sat I) and 5S RNA genes (5S) are indicated. Lane 1 shows the transcription of naked duplex DNA added to the egg extract and lane 2 shows markers (end-labeled *MspI* digest of pBR322 DNA). Phosphorimager quantitation of Sat I and 5S transcripts were 130 units (lane 3), 283 units (lane 4), 400 units (lane 5) and 2316 units (lane 6).

linker histone deficiency and the maintenance of histone acetylation states also contribute to facilitating transcription from chromatin assembled under these conditions (see also Figures 1 and 5).

Discussion

The major conclusion from this work is that acetylation of the core histones facilitates transcription of chromatin templates in the absence of linker histones (Figures 1, 5 and 6). Incorporation of linker histones into chromatin represses transcription independently of core histone acetylation (Figures 5 and 6). We have also excluded two molecular mechanisms by which acetylation of the core histones might facilitate transcription. Neither nucleosome mobility nor nucleosome positioning change as a consequence of histone acetylation (Figures 2–4). We suggest that local changes in the stability with which the N-terminal tails of the core histones contact DNA in the nucleosome dependent on their acetylation (Hong *et al.*, 1993) will have the major influence on transcription within these model templates.

Histone acetylation facilitates transcription

Histone acetylation has long been correlated with transcriptional activity (Allfrey *et al.*, 1964; Hebbes *et al.*, 1988; reviewed by Turner, 1991, 1993). An important and unresolved issue has been whether the acetylation of core histones could have a causal influence on transcription, or whether the accumulation of acetylated histones in chromatin was a consequence of transcriptional activity. Three lines of experimental work now suggest that histone acetylation has a causal role in facilitating the transcription process. (i) Histone acetylation can occur in genes that will become transcriptionally competent, but are yet to be transcribed (Hebbes *et al.*, 1988, 1992, 1994). (ii) Histone acetylation, in particular that of H4 (Vettese-Dadey *et al.*, 1996), facilitates transcription factor access to nucleosomal DNA (Lee *et al.*, 1993; Vettese-Dadey *et al.*, 1996) and the transcription of nucleosomal templates *in vitro* (Figures 1, 5 and 6). (iii) An increasing number of transcriptional activators have been characterized that have the capacity to acetylate the core histones (Brownell *et al.*, 1996; Mizzen *et al.*, 1996; Ogryzko *et al.*, 1996; Yang *et al.*, 1996). Although it is probable that these transcriptional activators have other functions that facilitate transcription (Barlev *et al.*, 1995), modification of the histones is established as functioning to increase the accessibility of nucleosomal DNA to the transcriptional machinery and provides a viable regulatory step for the transcription process (Brownell *et al.*, 1996; Wolffe and Pruss, 1996).

In this work, we have shown that the assembly of histone octamers onto a dinucleosomal template represses transcription, but that acetylation of the core histones can substantially relieve this repression (Figures 1, 5 and 6). How might histone acetylation facilitate transcription? Nucleosome mobility has been proposed as a molecular mechanism that permits the remodeling of repressive chromatin structures to allow gene activation (Ura *et al.*, 1995; Varga-Weisz *et al.*, 1995). Through a related mechanism, it is possible that histone acetylation might alter the exact distribution of histone octamers with respect to gene sequence such that key regulatory elements become

accessible to the transcriptional machinery (Ura *et al.*, 1995). Our experimental results (Figures 2, 3 and 4) indicate that acetylation of the core histones does not influence nucleosome mobility, and is without effect on the translational or rotational positioning of DNA with respect to the histone octamer. Earlier work has shown that mononucleosomes containing acetylated core histones and the 5S rRNA gene become more accessible to the gene-specific transcription factor TFIIIA (Lee *et al.*, 1993). Although TFIIIA could gain access to the 5S rRNA gene, the DNA templates used in these experiments were too short to be transcribed efficiently (Wolffe *et al.*, 1986). However, the increase in access of a transcription factor to mononucleosomal DNA is consistent with acetylation of the core histones facilitating transcription without a dependence on moving the histone octamer to alternate transcriptional positions, since on a short DNA template (186 bp, Wolffe *et al.*, 1986) there are only limited opportunities for the histone octamer to move to alternative translational positions. Acetylation of the H4 N-terminal tail renders the tail much more mobile in a nucleosomal context and greatly reduces its affinity for DNA in isolation (Cary *et al.*, 1982; Hong *et al.*, 1993). However, the stimulation of TFIIIA binding to the 5S nucleosome by histone acetylation is not dependent on the precise positioning of the histone octamer with respect to DNA sequence (Lee *et al.*, 1993), nor is the stimulation of Gal4 binding to nucleosomal DNA by H4 acetylation dependent on nucleosome position (Vettese-Dadey *et al.*, 1996). Thus it is possible that acetylation might exert a general destabilizing influence on histone–DNA interactions in the nucleosome.

Levels of histone acetylation such as those used in our experiments lead to a reduction in linking number change per 5S nucleosome *in vitro* (Norton *et al.*, 1989, 1990). This reduction in linker number occurs without changing the helical periodicity of DNA on the surface of the histone octamer (Bauer *et al.*, 1994). This suggests that the writhe of DNA in the nucleosome changes when the histones are acetylated, consistent with an allosteric change in nucleosome conformation. Others have suggested that the integrity of histone–histone interactions within the nucleosome is reduced following acetylation of the N-terminal tails *in vivo* (Chen and Allfrey, 1987; Chen *et al.*, 1990; Oliva *et al.*, 1990). However, rigorous biophysical studies indicate that histone hyperacetylation alone does not destabilize nucleosomes substantially *in vitro* (Ausio and van Holde, 1986; Garcia-Ramirez *et al.*, 1995). We suggest that the core histone tails have a major role in restricting transcription and that their acetylation can release this restriction through a local transition in nucleosome structure without alterations in the DNA sequences bound by individual histone octamers. Acetylation of the histone tails may be a necessary step in a staged process that requires additional histone modifications or events to destabilize the nucleosome further *in vivo*.

Linker histones repress transcription of nucleosomal templates independently of histone acetylation

Acetylated core histones are generally associated with transcriptionally active chromatin (Allegra *et al.*, 1987; Hebbes *et al.*, 1988), which is also relatively deficient in

histone H1 (Kamakaka and Thomas, 1990; Tazi and Bird, 1990; Bresnick *et al.*, 1992). Since the stability of chromatin higher order structures is dependent on both the core histone tails (Allan *et al.*, 1982; Annunziato and Seale, 1983; Garcia-Ramirez *et al.*, 1992) and the linker histone H1 (Thomas, 1984), it has been suggested that histone acetylation and histone H1 deficiency might act in concert to destabilize any higher order structure. Such an effect would be augmented further if core histone acetylation itself destabilizes the association of linker histone with chromatin (Ridsdale *et al.*, 1990; Perry and Annunziato, 1991; Juan *et al.*, 1994). However, in earlier work using mononucleosomes containing the 5S rRNA gene, we have found that histone H5 associates with nucleosomal DNA independently of histone acetylation status (Ura *et al.*, 1994). Here we extend these observations to demonstrate that histone H1 binds efficiently to dinucleosomes reconstituted with either acetylated or control core histones (Figure 5A) and represses transcription (Figure 5B). Our results indicate that histone acetylation in isolation does not severely restrict the incorporation of linker histones in chromatin. It is possible that other proteins that recognize acetylated core histones such as the small subunit of CAF1 (p48, Verreault *et al.*, 1996), might be stably incorporated into nascent acetylated chromatin such that histone H1 is excluded. In our model dinucleosome, incorporation of histone H1 restricts the translational mobility of both acetylated and control nucleosomes, without influencing the rotational positioning of DNA on the surface of the histones (data not shown). Restriction of the mobility of histone octamers by histone H1 can account for the observed repression of transcription (Ura *et al.*, 1995).

The repression of transcription mediated by histone H1 on acetylated templates implies that removal of H1 will still be a necessary component of any transcriptional activation process in which targeted core histone acetylation is involved.

A role for histone acetylation during chromatin assembly in facilitating transcription

Our experiments with acetylated histones and model dinucleosome templates provide some insight into the consequences of the acetylation of pre-formed nucleosome structures for transcription. Such regulated acetylation might be directed by targeted components of the transcriptional machinery (Brownell *et al.*, 1996; Mizzen *et al.*, 1996; Ogryzko *et al.*, 1996; Yang *et al.*, 1996). However, a predominant form of histone acetylation during S phase of the cell cycle is that associated with the assembly of nascent chromatin (Ruiz-Carrillo *et al.*, 1975; Parthun *et al.*, 1996; Verreault *et al.*, 1996). In particular, histone H4 is diacetylated on lysines 5 and 12 (Sobel *et al.*, 1995) prior to nucleosome assembly and then deacetylated as chromatin matures (Jackson *et al.*, 1976; Shimamura and Worcel, 1989). Inhibition of deacetylation using sodium butyrate (Figure 6A) augments transcription >10-fold in the absence of linker histones (Figure 6E). The significance of the capacity of histone acetylation to potentiate the transcriptional competence of chromatin under linker histone-deficient conditions is that it provides a potential mechanism for the programming of genes during replication-coupled chromatin assembly (Lee *et al.*, 1993; Verreault

et al., 1996). Since nucleosome assembly is staged *in vivo* with the initial deposition into stable nucleoprotein complexes of histones H3/H4, followed by H2A/H2B and finally linker histones, such as B4 and H1 (Worcel *et al.*, 1978), intermediate chromatin structures enriched in acetylated histones but deficient in linker histones will be relatively accessible to components of the transcriptional machinery. This would provide a window of opportunity to pre-assemble preset chromatin configurations competent for subsequent transcription (Becker, 1994; Wallrath *et al.*, 1994).

Materials and methods

Plasmids and DNA fragments

The construction of the pX5S 197-2 plasmid containing two tandem repeats of the 5S RNA gene has been described (Ura *et al.*, 1995). A 424 bp *Xba*I–*Xho*I fragment derived from plasmid pX5S 197-2 was isolated from non-denaturing acrylamide gels for nucleosome reconstitution after end-labeling at the *Xba*I site with T7 polynucleotide kinase or Klenow fragment (New England Biolabs). This fragment was reconstituted into nucleosomes. Single-stranded DNA from bacteriophage M13 mp18 and class III gene derivatives was prepared as previously described (Almouzni and Méchali, 1988a,b; Almouzni *et al.*, 1990a,b).

Nucleosome reconstitution

Chromatin was purified from HeLa cells according to Lee *et al.* (1993). Nuclei isolated from cells treated with or without 10 mM sodium butyrate for 24 h were sonicated extensively, producing chromatin lengths of 10–30 nucleosomes. Purified chromatin was treated with Dowex 50 W-X2 in 0.2 M NaCl, 0.05 M potassium phosphate (pH 6.8) to remove linker histone H1 (Thoma *et al.*, 1979). Nucleosome cores were reconstituted onto radiolabeled DNA fragments by the histone exchange method (Tatchell and van Holde, 1977). A 15-fold template mass excess of chromatin was mixed with radiolabeled DNA in tubes followed by slow adjustment of the NaCl concentration (to 1 M). Tubes were incubated at 37°C for 15 min. Samples were transferred to a dialysis bag (with a molecular size limit of 6–8 kDa) and dialyzed against 1.0 M NaCl, 10 mM Tris–HCl (pH 7.5), 1 mM EDTA, 0.1 mM 2-mercaptoethanol overnight. In this case, almost all products were dinucleosome cores and no naked DNA fragment or mononucleosome cores were detectable by nucleoprotein agarose electrophoresis.

After reconstitution, the oligonucleosome cores were loaded on 5–20% sucrose gradients containing 10 mM Tris–HCl (pH 7.5) 1 mM EDTA and 0.1 mM phenylmethylsulfonyl fluoride (PMSF), and then centrifuged for 16 h at 35 000 r.p.m. at 4°C in a Beckman SW41 rotor. Fractions were collected and analyzed by nucleoprotein agarose (0.7%) gel in 0.5× TBE (1× TBE is 90 mM Tris base/90 mM boric acid/2.5 mM EDTA). Fractions containing mono-, di- or trinucleosomes were pooled separately, concentrated to ~2.5 µg/ml using microcon-30 (amicon), and dialyzed against 10 mM Tris–HCl (pH 7.5), 0.1 mM EDTA, 1 mM 2-mercaptoethanol overnight at 4°C. Samples were stored on ice until use. Chicken erythrocyte oligonucleosomes (chromatin lengths of 1–30 nucleosomes) were prepared after removal of linker histones (Lee *et al.*, 1993), and used as a control for isolation of the native dinucleosome complex.

Analysis of histone modification

Histones were resolved both on SDS–18% polyacrylamide gel (Laemmli, 1970) and on Triton acid–urea–15% polyacrylamide gels (Zweidler, 1978). Triton acid–urea gels were modified from the published protocol to optimize resolution (19:1 15% acrylamide:bisacrylamide, 6.3 M urea, 7 mM Triton X-100, 5% acetic acid). Gels were pre-run for 2–3 h with a top buffer layer of 6.3 M urea, 7 mM Triton X-100 containing 0.5 M 2-aminoethanethiol-HCl (Aldrich) as a scavenger. Electrophoresis was carried out in 5% acetic acid with an applied constant current of 3 mA/cm in a buffer-cooled electrophoresis tank for ~5 h. Analytic gels subsequently were fixed and stained with Coomassie blue. Proteins from Triton acid–urea gels were electrotransferred onto nitrocellulose and stained with Ponceau S (Sigma) to evaluate transfer efficiency. Western blotting and immunochemical staining with polyclonal antibodies for the acetylated isoform of histone H4 (Turner and Fellows, 1989; Turner *et al.*, 1992) was as previously described (Dimitrov *et al.*, 1994; O'Neill

and Turner, 1995). Two-dimensional electrophoresis was performed as described (Russanova *et al.*, 1980, 1989). The proteins were first separated in a 15% polyacrylamide slab gel containing 7 M urea and 5% acetic acid (Panyim and Chalkley, 1969). The strip with the separated proteins was then cut out from the gel and placed on the top of a second gel, which was made of a 2–3 cm 5% stacking gel and a 12–15 cm separating gel, containing 0.4% Triton X-100 and 6 M urea (West and Bonner, 1980). The gels were stained either with 0.1% Coomassie brilliant blue R-250 (Bio-Rad Laboratories, Cambridge, MA) or with silver nitrate as described (Wray *et al.*, 1981).

H1 binding experiments

A total of 100 ng (DNA content) of reconstituted nucleosome cores (0.6 pmol) were incubated with various amounts of histone H1 in 10 µl of binding buffer [10 mM Tris–HCl (pH 8.0), 50 mM NaCl, 0.1 mM EDTA, 5% (v/v) glycerol] at room temperature for 15–30 min (Hayes and Wolffe, 1993). Samples were loaded directly onto running 0.7% agarose gel in 0.5× TBE. After electrophoresis, the gels were dried and autoradiographed.

Two-dimensional gel experiments

Two-dimensional gel experiments to show the redistribution of nucleosome cores were performed following the procedure of Meersseman *et al.* (1992) with slight modifications. Reconstitutes with or without histone H1 were loaded onto non-denaturing 4% polyacrylamide (29:1 acrylamide:bisacrylamide) gels at 4°C in 0.5× TBE. The gels were run at a maximum of 10 V/cm. Each lane was cut in half lengthwise. One half of each lane was left at 4°C, and the other was sealed and immersed at 37°C for 1 h. The gel strips were then arranged on top of a second non-denaturing gel in the cold, and the second dimension was electrophoresed at 4°C under the same conditions as the first dimension.

Hydroxyl radical footprinting of dinucleosomes

Reconstitutes with or without histone H1 were treated with hydroxyl radicals prior to resolving nucleoprotein complexes on preparative 0.7% agarose gels (Hayes and Wolffe, 1992b). Samples contained labeled dinucleosome (60 ng of DNA) and chicken erythrocyte core particles (~1 µg) and were incubated with or without 200 ng of histone H1 (one molecule/nucleosome core) as described above. After electrophoresis, bound or unbound H1 dinucleosome complexes were excised from the gel. DNA from these complexes was isolated and analyzed by denaturing polyacrylamide (6%) gel electrophoresis. Specific DNA markers were produced by Maxam and Gilbert cleavage at G residues.

Micrococcal nuclease mapping of dinucleosomes

Dinucleosomes (80 ng of DNA) in the absence or presence of 16 ng of histone H1 (molar ratio of histone to DNA = 1) were digested with 0.075–0.6 U of micrococcal nuclease (Pharmacia) for 5 min at 22°C. Incubation with H1 was as described above. Ca^{2+} was adjusted to 0.5 mM concomitantly with addition of micrococcal nuclease. Digestions were terminated with addition of EDTA (5 mM), SDS (0.25%, w/v) and proteinase K (Gibco BRL) (1 mg/ml). The DNA was recovered and 5'-end-labeled with [γ - ^{32}P]ATP and T4 polynucleotide kinase, and the end-labeled DNA fragments were separated by electrophoresis in non-denaturing 6% polyacrylamide gels. DNA fragments of nucleosome core and chromatosome products were recovered and digested with restriction endonucleases to determine micrococcal nuclease cleavage sites (Hayes and Wolffe, 1993).

Transcription reactions for dinucleosomes

Mono-, di- or trinucleosome complexes previously resolved and separated by sucrose gradient centrifugation or naked DNA were used as templates for transcription in an extract from *Xenopus* oocyte nuclei. Oocyte nuclear extract was prepared as described previously (Birkenmeier *et al.*, 1978). Transcription reaction conditions were as follows: 10 ng of radiolabeled template were added to a 10 µl reaction mixture containing 5 µl of nuclear extract in J buffer [10 mM HEPES (pH 7.4), 50 mM DCl, 7 mM MgCl_2 , 2.5 mM dithiothreitol (DTT), 0.25 U/µl RNasin (Gibco/BRL) and 0.1 mM EDTA] and pre-incubated for 20 min before addition of exogenous triphosphates to 250 µM ATP, CTP and GTP, 50 µM UTP with 2.5 µCi of added [α - ^{32}P]UTP. The reaction temperature was 22°C. Labeling was continued for 40 min after pre-incubation. Radiolabeled transcripts were extracted with phenol, precipitated with ethanol and analyzed by electrophoresis in a 6% denaturing polyacrylamide gel. The level of 5S RNA transcription was quantitated with a Molecular Dynamics PhosphorImager. The radiolabeled 5S DNA template served as an internal control for recovery.

Egg collection and preparation of egg extract

Xenopus laevis frogs were purchased from Xenopus I, Ann Arbor, Michigan. Unfertilized eggs were collected from *X. laevis* frogs by injection of chorionic gonadotropin (Pregnyl; Organon). Eggs were collected in modified high-salt Barth saline, dejellied with 2% cysteine in 1/10 Barth saline (pH 7.9), rinsed and sorted under a dissecting microscope to remove all damaged or abnormal eggs. The egg extract was prepared at 4°C as described (Almouzni and Méchali, 1988a,b). The eggs were washed in extraction medium [20 mM HEPES (pH 7.5), 70 mM potassium chloride, 1 mM DTT, 5% glycerol] and packed in centrifuge tubes. Excess medium was removed, leaving only the interstitial buffer between the cells. The cells were broken by direct centrifugation (12 000 g for 30 min), and the supernatant was collected. The supernatant was then centrifuged at 150 000 g for 60 min in a Beckman 50 Ti rotor. The extract was stored in small aliquots at 80°C. Extracts were depleted of histone B4 as previously described (Dimitrov *et al.*, 1994).

DNA synthesis, chromatin assembly and transcription reactions in egg extract

Unless otherwise specified, our standard reaction mixtures contained 10–20 µg of DNA per ml of egg extract supplemented with exogenous 3 mM ATP and 5 mM MgCl₂. We added 10–15 µCi of [α -³²P]dATP per ml to monitor the DNA synthesis. Unless otherwise specified, chromatin assembly reactions were digested by micrococcal nuclease after addition of 3 mM CaCl₂. Aliquots were taken during digestion, made up to 30 mM EDTA and 0.5% SDS, and treated as described previously (Almouzni *et al.*, 1991), either for gel electrophoresis or for counting of acid-insoluble material. When subjected to electrophoresis, the samples were deproteinized by phenol extraction after proteinase I (500 µg/ml) treatment. Each transcription reaction contained duplex DNA at a final concentration of 10–20 µg/ml, 500 µM ATP, 500 µM UTP, 500 µM CTP, 100 µM GTP, 10 µCi of [α -³²P]GTP (3000 Ci/mmol) and 5 U of human placental RNase inhibitor (Bethesda Research Laboratories). Reactions were incubated for the times indicated. The samples were then processed for sequencing gel analysis as previously described (Almouzni *et al.*, 1991).

Acknowledgements

We thank Dr Bryan Turner (University of Birmingham, UK) for the kind gift of antibodies specific for histone H4 isoforms. We thank Ms Thuy Vo for manuscript preparation.

References

- Allan, J., Harborne, N., Rau, D.C. and Gould, H. (1982) Participation of the core histone 'tails' in the stabilization of the chromatin solenoid. *J. Cell Biol.*, **93**, 285–297.
- Allegra, P., Sterner, R., Clayton, D.F. and Allfrey, V.G. (1987) Affinity chromatographic purification of nucleosomes containing transcriptionally active DNA sequences. *J. Mol. Biol.*, **196**, 379–388.
- Allfrey, V., Faulkner, R.M. and Mirsky, A.E. (1964) Acetylation and methylation of histones and their possible role in the regulation of RNA synthesis. *Proc. Natl Acad. Sci. USA*, **51**, 786–794.
- Almouzni, G. and Méchali, M. (1988a) Assembly of spaced chromatin promoted by DNA synthesis in extracts from *Xenopus* eggs. *EMBO J.*, **7**, 664–672.
- Almouzni, G. and Méchali, M. (1988b) Assembly of spaced chromatin: involvement of ATP and DNA topoisomerase activity. *EMBO J.*, **7**, 4355–4365.
- Almouzni, G., Clark, D.J., Méchali, M. and Wolffe, A.P. (1990a) Chromatin assembly on replicating DNA *in vitro*. *Nucleic Acids Res.*, **18**, 5767–5774.
- Almouzni, G., Méchali, M. and Wolffe, A.P. (1990b) Competition between transcription complex assembly and chromatin assembly on replicating DNA. *EMBO J.*, **9**, 573–582.
- Almouzni, G., Méchali, M. and Wolffe, A.P. (1991) Transcription complex disruption caused by a transition in chromatin structure. *Mol. Cell. Biol.*, **11**, 655–665.
- Annunziato, A.T. and Seale, R.L. (1983) Histone deacetylation is required for the maturation of newly replicated chromatin. *J. Biol. Chem.*, **258**, 12675–12684.
- Ausio, J. and van Holde, K.E. (1986) Histone hyperacetylation: its effects on nucleosome conformation and stability. *Biochemistry*, **25**, 1421–1428.
- Barlev, N.A., Candau, R., Wang, L., Darpino, P., Silverman, N. and Berger, S.L. (1995) Characterization of physical interactions of the putative transcriptional adaptor ADA2 with acidic activation domains and TATA-binding-protein. *J. Biol. Chem.*, **270**, 19337–19334.
- Bartsch, J., Truss, M., Bode, J. and Beato, M. (1996) Moderate increase in histone acetylation activates the mouse mammary tumor virus promoter and remodels its nucleosome structure. *Proc. Natl Acad. Sci. USA*, **93**, 10741–10745.
- Bauer, W.R., Hayes, J.J., White, J.H. and Wolffe, A.P. (1994) Nucleosome structural changes due to acetylation. *J. Mol. Chem.*, **236**, 685–690.
- Becker, P.B. (1994) The establishment of active promoters in chromatin. *BioEssays*, **16**, 541–547.
- Birkenmeier, E.H., Brown, D.D. and Jordan, E. (1978) A nuclear extract of *Xenopus laevis* oocytes that accurately transcribes 5S RNA genes. *Cell*, **15**, 1077–1086.
- Bogenhagen, D.F., Wormington, W.M., and Brown, D.D. (1982) Stable transcription complexes of *Xenopus* 5S RNA genes: a means to maintain the differentiated state. *Cell*, **28**, 413–421.
- Braunstein, M., Rose, A.B., Holmes, S.G., Allis, C.D. and Broach, J.R. (1993) Transcriptional silencing in yeast is associated with reduced histone acetylation. *Genes Dev.*, **7**, 592–604.
- Bresnick, E.H., Bustin, M., Marsaud, V., Richard-Foy, H. and Hager, G.L. (1992) The transcriptionally-active MMTV promoter is depleted of H1. *Nucleic Acids Res.*, **20**, 373–378.
- Brownell, J.E., Zhou, J., Ranalli, T., Kobayashi, R., Edmondson, D.G., Roth, S.Y. and Allis, C.D. (1996) *Tetrahymena* histone acetyltransferase A: a homolog to yeast Gcn5p linking histone acetylation to gene activation. *Cell*, **84**, 843–851.
- Cary, P.D., Crane-Robinson, C., Bradbury, E.M. and Dixon, G.H. (1982) Effect of acetylation on the binding of N-terminal peptides of histone H4 to DNA. *Eur. J. Biochem.*, **127**, 137–143.
- Chen, H., Li, B. and Workman, J.L. (1994) A histone-binding protein nucleoplasmin stimulates transcription factor binding to nucleosomes and factor-induced nucleosome disassembly. *EMBO J.*, **13**, 380–390.
- Chen, T.A. and Allfrey, V.G. (1987) Rapid and reversible changes in nucleosome structure accompany the activation, repression and super induction of the murine proto-oncogenes *c-fos* and *c-myc*. *Proc. Natl Acad. Sci. USA*, **84**, 5252–5256.
- Chen, T.A., Sterner, R., Cozzolino, A. and Allfrey, V.G. (1990) Reversible and irreversible changes in nucleosome structure along the *c-fos* and *c-myc* oncogenes following inhibition of transcription. *J. Mol. Biol.*, **212**, 481–493.
- Dasso, M., Dimitrov, S. and Wolffe, A.P. (1994) Nuclear assembly is independent of linker histones. *Proc. Natl Acad. Sci. USA*, **91**, 12477–12481.
- Dimitrov, S., Almouzni, G., Dasso, M. and Wolffe, A.P. (1993) Chromatin transitions during early *Xenopus* embryogenesis: changes in histone H4 acetylation and in linker histone type. *Dev. Biol.*, **160**, 214–227.
- Dimitrov, S., Dasso, M.C. and Wolffe, A.P. (1994) Remodeling sperm chromatin in *Xenopus laevis* egg extracts: the role of core histone phosphorylation and linker histone B4 in chromatin assembly. *J. Cell Biol.*, **126**, 591–601.
- Durrin, L.K., Mann, R.K., Kayne, P.S. and Grunstein, M. (1991) Yeast histone H4 N-terminal sequence is required for promoter activation *in vivo*. *Cell*, **65**, 1023–1031.
- Felsenfeld, G. (1992) Chromatin: an essential part of the transcriptional apparatus. *Nature*, **355**, 219–224.
- Fisher-Adams, G. and Grunstein, M. (1995) Yeast histone H4 and H3 N-termini have different effects on the chromatin structure of the *Gall* promoter. *EMBO J.*, **14**, 1468–1477.
- Garcia-Ramirez, M., Dong, F. and Ausio, J. (1992) Role of the histone 'tails' in the folding of oligonucleosomes depleted of histone H1. *J. Biol. Chem.*, **267**, 19587–19595.
- Garcia-Ramirez, M., Rocchini, C. and Ausio, J. (1995) Modulation of chromatin folding by histone acetylation. *J. Biol. Chem.*, **270**, 17923–17928.
- Godde, J.S., Nakatani, Y. and Wolffe, A.P. (1995) The amino-terminal tails of the core histones and the translational position of the TATA box determine TBP/TFIIA association with nucleosomal DNA. *Nucleic Acids Res.*, **23**, 4557–4564.
- Grunstein, M., Durrin, L.K., Mann, R.K., Fisher-Adams, G. and Johnson, L.M. (1992) Histones: regulators of transcription in yeast. In McKnight, S. and Yamamoto, K. (eds), *Transcriptional Regulation*. Cold Spring Harbor Laboratory Press, Cold Spring Harbor, NY, pp. 1295–1315.

- Hansen, J.C. and Wolffe, A.P. (1992) Chromatin folding inhibits both transcription initiation and elongation by RNA polymerase III. *Biochemistry*, **31**, 7977–7988.
- Hayes, J.J. and Wolffe, A.P. (1992a) The interaction of transcription factors with nucleosomal DNA. *BioEssays*, **14**, 597–603.
- Hayes, J.J. and Wolffe, A.P. (1992b) Histones H2A/H2B inhibit the interactions of transcription factor IIIA with the *Xenopus borealis* somatic 5S RNA gene in a nucleosome. *Proc. Natl Acad. Sci. USA*, **89**, 1229–1233.
- Hayes, J.J. and Wolffe, A.P. (1993) Preferential and asymmetric interaction of linker histones with 5S DNA in the nucleosome. *Proc. Natl Acad. Sci. USA*, **90**, 6415–6419.
- Hayes, J.J., Kaplan, R., Ura, K., Pruss, D. and Wolffe, A.P. (1996) Basic residues within a putative DNA-binding surface in the globular domain of linker histones are not essential for specific binding to the nucleosome core. *J. Biol. Chem.*, **271**, 25817–25822.
- Hebbes, T.R., Thorne, A.W. and Crane-Robinson, C. (1988) A direct link between core histone acetylation and transcriptionally active chromatin. *EMBO J.*, **7**, 1395–1402.
- Hebbes, T.R., Thorne, A.W., Clayton, A.L. and Crane-Robinson, C. (1992) Histone acetylation and globin gene switching. *Nucleic Acids Res.*, **20**, 1017–1022.
- Hebbes, T.R., Clayton, A.L., Thorne, A.W. and Crane-Robinson, C. (1994) Core histone hyperacetylation co-maps with generalized DNase I sensitivity in the chicken β -globin chromosomal domain. *EMBO J.*, **13**, 1823–1830.
- Hong, L., Schroth, G.P., Matthews, H.R., Yau, P. and Bradbury, E.M. (1993) Studies of the DNA binding properties of the histone H4 amino terminus. *J. Biol. Chem.*, **268**, 305–314.
- Imbalzano, A.M., Kwon, H., Green, M.R. and Kingston, R.E. (1994) Facilitated binding of TATA-binding protein to nucleosomal DNA. *Nature*, **370**, 481–485.
- Jackson, V., Shires, A., Tanphaichitr, N. and Chalkley, R. (1976) Modification of histones immediately after synthesis. *J. Mol. Biol.*, **104**, 471–483.
- Juan, L.J., Utley, R., Adams, C.C., Vettese-Dadey, M. and Workman, J.L. (1994) Differential repression of transcription factor binding by histone H1 is regulated by the core histone amino termini. *EMBO J.*, **13**, 6031–6040.
- Kamakaka, R.T. and Thomas, J.O. (1990) Chromatin structure of transcriptionally competent and repressed genes. *EMBO J.*, **9**, 3997–4006.
- Kayne, P.S., Kim, U.J., Han, M., Mullen, J.R., Yoshizaki, F. and Grunstein, M. (1988) Extremely conserved histone H4 N-terminus is dispensable for growth but essential for repressing the silent mating loci in yeast. *Cell*, **55**, 27–39.
- Laemmli, U.K. (1970) Cleavage of structural proteins during the assembly of the head of bacteriophage T4. *Nature*, **227**, 680–685.
- Lee, D.Y., Hayes, J.J., Pruss, D. and Wolffe, A.P. (1993) A positive role for histone acetylation in transcription factor binding to nucleosomal DNA. *Cell*, **72**, 73–84.
- Lorch, Y., La Pointe, J.W. and Kornberg, R.D. (1987) Nucleosomes inhibit the initiation of transcription but allow chain elongation with the displacement of histones. *Cell*, **49**, 203–210.
- Meersseman, G., Pennings, S. and Bradbury, E.M. (1991) Chromatosome positioning on assembled long chromatin: linker histones affect nucleosome placement on 5S DNA. *J. Mol. Biol.*, **220**, 89–100.
- Meersseman, G., Pennings, S. and Bradbury, E.M. (1992) Mobile nucleosomes—a general behavior. *EMBO J.*, **11**, 2951–2959.
- Mizzen, C.A. et al. (1996) The TAF_{II}250 subunit of TFIID has histone acetyltransferase activity. *Cell*, **87**, 1261–1270.
- Nightingale, K., Dimitrov, S., Reeves, R. and Wolffe, A.P. (1996a) Evidence for a shared structural role for HMG1 and linker histones B4 and H1 in organizing chromatin. *EMBO J.*, **15**, 548–561.
- Nightingale, K.P., Pruss, D. and Wolffe, A.P. (1996) A single high affinity binding site for histone H1 in a nucleosome containing the *Xenopus borealis* 5S ribosomal RNA gene. *J. Biol. Chem.*, **271**, 7090–7094.
- Norton, V.G., Imai, B.S., Yau, P. and Bradbury, E.M. (1989) Histone acetylation reduces nucleosome core particle linking number change. *Cell*, **57**, 449–457.
- Norton, V.G., Marvin, K.W., Yau, P. and Bradbury, E.M. (1990) Nucleosome linking number change controlled by acetylation of histones H3 and H4. *J. Biol. Chem.*, **265**, 19848–19852.
- O'Neill, L.P. and Turner, B.M. (1995) Histone H4 acetylation distinguishes coding regions of the human genome from heterochromatin in a differentiation-dependent but transcription-independent manner. *EMBO J.*, **14**, 3946–3957.
- Ogryzko, V.V., Schiltz, R.L., Russanova, V., Howard, B.H. and Nakatani, Y. (1996) The transcriptional coactivators p300 and CBP are histone acetyltransferases. *Cell*, **87**, 953–959.
- Oliva, R., Bazett-Jones, D.P., Locklear, L. and Dixon, G.H. (1990) Histone hyperacetylation can induce unfolding of the nucleosome core particle. *Nucleic Acids Res.*, **18**, 2739–2747.
- Owen-Hughes, T. and Workman, J.L. (1994) Experimental analysis of chromatin function in transcriptional control. *Crit. Rev. Euk. Gene Expr.*, **4**, 1–39.
- Panyim, S. and Chalkley, R. (1969) The heterogeneity of histone I. A quantitative analysis of calf histones in very long polyacrylamide gels. *Biochemistry*, **8**, 3972–3979.
- Parthun, M.R., Widom, J. and Gottschling, D.E. (1996) The major cytoplasmic histone acetyltransferase in yeast: links to chromatin replication and histone metabolism. *Cell*, **87**, 85–94.
- Pennings, S., Meersseman, G. and Bradbury, E.M. (1994) Linker histones H1 and H5 prevent the mobility of positioned nucleosomes. *Proc. Natl Acad. Sci. USA*, **91**, 10275–10279.
- Perlmann, T. and Wrangé, O. (1988) Specific glucocorticoid receptor binding to DNA reconstituted in a nucleosome. *EMBO J.*, **7**, 3073–3083.
- Perry, C.A. and Annunziato, A.T. (1989) Influence of histone acetylation on the solubility, H1 content and DNase I sensitivity of newly replicated chromatin. *Nucleic Acids Res.*, **17**, 4275–4291.
- Perry, C.A. and Annunziato, A.T. (1991) Histone acetylation reduces H1-mediated nucleosome interactions during chromatin assembly. *Exp. Cell Res.*, **196**, 337–345.
- Ridsdale, J.A., Henzdel, M.F., Decluve, G.P. and Davie, J.R. (1990) Histone acetylation alters the capacity of the H1 histones to condense transcriptionally active/competent chromatin. *J. Biol. Chem.*, **265**, 5150–5156.
- Ruiz-Carrillo, A., Wangh, L.J. and Allfrey, V.G. (1975) Processing of newly synthesized histone molecules. *Science*, **190**, 117–128.
- Russanova, V., Venkov, C. and Tsanev, R. (1980) A comparison of histone variants in different rat tissues. *Cell Differ.*, **9**, 339–350.
- Russanova, V., Stephanova, E., Pashev, I. and Tsanev, R. (1989) Histone variants in mouse centromeric chromatin. *Mol. Cell. Biochem.*, **90**, 1–7.
- Schild, C., Claret, F.-X., Wahli, W. and Wolffe, A.P. (1993) A nucleosome-dependent static loop potentiates estrogen-regulated transcription from the *Xenopus* vitellogenin B1 promoter *in vitro*. *EMBO J.*, **12**, 423–433.
- Shen, X. and Gorovsky, M.A. (1996) Linker histone H1 regulates specific gene expression but not global transcription *in vivo*. *Cell*, **86**, 475–483.
- Shimamura, A. and Worcel, A. (1989) The assembly of regularly spaced nucleosomes in the *Xenopus* oocyte S150 extract is accompanied by deacetylation of histone H4. *J. Biol. Chem.*, **264**, 14524–14530.
- Simpson, R.T. (1991) Nucleosome positioning: occurrence, mechanisms and functional consequences. *Progr. Nucleic Acids Mol. Biol.*, **40**, 143–184.
- Sobel, R.E., Cook, R.G., Perry, C.A., Annunziato, A.T. and Allis, C.D. (1995) Conservation of deposition-related acetylation sites in newly synthesized histones H3 and H4. *Proc. Natl Acad. Sci. USA*, **92**, 1237–1241.
- Svaren, J. and Horz, W. (1996) Regulation of gene expression by nucleosomes. *Curr. Opin. Genet. Dev.*, **6**, 164–170.
- Svaren, J. and Horz, W. (1995) Interplay between nucleosomes and transcription factors at the yeast PHO5 promoter. *Semin. Cell Biol.*, **6**, 177–183.
- Tatchell, K. and van Holde, K.E. (1977) Reconstitution of chromatin core particles. *Biochemistry*, **16**, 5295–5303.
- Tazi, J. and Bird, A. (1990) Alternative chromatin structure at CpG islands. *Cell*, **60**, 902–920.
- Thoma, F., Koller, T. and Klug, A. (1979) Involvement of histone H1 in the organization of the nucleosome and of the salt dependent superstructures of chromatin. *J. Cell Biol.*, **83**, 403–427.
- Thomas, J.O. (1984) The higher order structure of chromatin and histone H1. *J. Cell Sci. Suppl.*, **1**, 1–20.
- Truss, M., Bartsch, J., Schelbert, A., Haché, R.J.G. and Beato, M. (1995) Hormone induces binding of receptors and transcription factors to a rearranged nucleosome on the MMTV promoter *in vivo*. *EMBO J.*, **14**, 1737–1751.
- Turner, B.M. (1991). Histone acetylation and control of gene expression. *J. Cell Sci.*, **99**, 13–20.
- Turner, B.M. (1993) Decoding the nucleosome. *Cell*, **75**, 5–8.
- Turner, B. and Fellows, G. (1989) Specific antibodies reveal ordered and cell cycle-related use of histone H4 acetylation sites in mammalian cells. *Eur. J. Biochem.*, **179**, 131–139.

- Turner, B.M. and O'Neill, L.P. (1995) Histone acetylation in chromatin and chromosomes. *Semin. Cell Biol.*, **6**, 229–236.
- Turner, B.M., Birley, A.J. and Lavender, J. (1992) Histone H4 isoforms acetylated at specific lysine residues define individual chromosomes and chromatin domains in *Drosophila* polytene nuclei. *Cell*, **69**, 375–384.
- Ura, K., Wolffe, A.P. and Hayes, J.J. (1994) Core histone acetylation does not block linker histone binding to a nucleosome including a *Xenopus borealis* 5S rRNA gene. *J. Biol. Chem.*, **269**, 27171–27174.
- Ura, K., Hayes, J.J. and Wolffe, A.P. (1995) A positive role for nucleosome mobility in the transcriptional activity of chromatin templates: restriction by linker histones. *EMBO J.*, **14**, 3752–3765.
- Ura, K., Nightingale, K. and Wolffe, A.P. (1996) Differential association of HMG1 and linker histones B4 and H1 with dinucleosomal DNA: structural transitions and transcriptional repression. *EMBO J.*, **15**, 4959–4969.
- Varga-Weisz, P.D., Blank, T.A. and Becker, P.B. (1995) Energy-dependent chromatin accessibility and nucleosome mobility in a cell free system. *EMBO J.*, **14**, 2209–2216.
- Verreault, A., Kaufman, P.D., Kobayashi, R. and Stillman, B. (1996) Nucleosome assembly by a complex of CAF-1 and acetylated histones H3/H4. *Cell*, **87**, 95–104.
- Vettese-Dadey, M., Walter, P., Chen, H., Juan, L.-J. and Workman, J.L. (1994) Role of the histone amino termini in facilitated binding of a transcription factor, GAL4-AH to nucleosome cores. *Mol. Cell. Biol.*, **14**, 970–981.
- Vettese-Dadey, M., Grant, P.A., Hebbes, R.T., Crane-Robinson, C., Allis, C.D. and Workman, J.L. (1996) Acetylation of histone H4 plays a primary role in enhancing transcription factor binding to nucleosomal DNA *in vitro*. *EMBO J.*, **15**, 2508–2518.
- Wallrath, L.L., Lu, Q., Granok, H. and Elgin, S.C.R. (1994) Architectural variations of inducible eukaryotic promoters: preset and remodeling chromatin structures. *BioEssays*, **16**, 165–170.
- West, M.H.P. and Bonner, W.M. (1980) Histone H2B can be modified by the attachment of ubiquitin. *Nucleic Acids Res.*, **8**, 4671–4680.
- Wolffe, A.P. (1989) Dominant and specific repression of *Xenopus* oocyte 5S RNA genes and satellite I DNA by histone H1. *EMBO J.*, **8**, 527–537.
- Wolffe, A.P. (1995) *Chromatin: Structure and Function*. 2nd edn. Academic Press, London.
- Wolffe, A.P. and Pruss, D. (1996) Targeting chromatin disruption: transcription factors that acetylate histones. *Cell*, **84**, 817–819.
- Wolffe, A.P., Jordan, E. and Brown, D.D. (1986) A bacteriophage RNA polymerase transcribes through a *Xenopus* 5S RNA gene transcription complex without disrupting it. *Cell*, **44**, 381–389.
- Wolffe, A.P., Andrews, M.T., Crawford, E.T., Losa, R.M. and Brown, D.D. (1987) Negative supercoiling is not required for 5S RNA transcription *in vitro*. *Cell*, **49**, 301–303.
- Wong, J., Shi, Y.-B. and Wolffe, A.P. (1995) A role for nucleosome assembly in both silencing and activation of the *Xenopus* TR β A gene by the thyroid hormone receptor. *Genes Dev.*, **9**, 2696–2711.
- Worcel, A., Han, S. and Wong, M.L. (1978) Assembly of newly replicated chromatin. *Cell*, **15**, 969–977.
- Wray, W., Boulikas, T., Wray, V. and Hancock, R. (1981) Silver staining of proteins in polyacrylamide gels. *Anal. Biochem.*, **118**, 197–203.
- Yang, X.J., Ogryzko, V.V., Nishikawa, J.H., Howard, B. and Nakatani, Y. (1996) A p300/CBP-associated factor that competes with the adenoviral E1A oncoprotein. *Nature*, **382**, 319–324.
- Yoshida, M., Horinouchi, S. and Beppu, T. (1995) Trichostatin A and trapoxin: novel chemical probes for the role of histone acetylation in chromatin structure and function. *BioEssays*, **17**, 423–430.
- Zweidler, A. (1978) Resolution of histones by polyacrylamide gel electrophoresis in the presence of nonionic detergent. *Methods Cell Biol.*, **17**, 223–233.

Received on November 25, 1996; revised on January 10, 1997