S-phase-dependent action of cycloheximide in relieving chromatin-mediated general transcriptional repression

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Chromatin plays a major role in the tight regulation of gene expression and in constraining inappropriate gene activity. Replication-coupled chromatin assembly ensures maintenance of these functions of chromatin during S phase of the cell cycle. Thus treatment of cells with an inhibitor of translation, such as cycloheximide (CX), would be expected to have a dramatic effect on chromatin structure and function, essentially in S phase of the cell cycle, due to uncoupled DNA replication and chromatin assembly. In this work, we confirm this hypothesis and show that CX can induce a dramatic S-phase-dependent alteration in chromatin structure that is associated with general RNA polymerase II-dependent transcriptional activation. Using two specific RNA polymerase II-transcribed genes, we confirm the above conclusion and show that CX-mediated transcriptional

activation is enhanced during the DNA replication phase of the cell cycle. Moreover, we show co-operation between an inhibitor of histone deacetylase and CX in inducing gene expression, which is again S-phase-dependent. The modest effect of CX in inducing the activity of a transiently transfected promoter shows that the presence of the promoter in an endogenous chromatin context is necessary in order to observe transcriptional activation. We therefore suggest that the uncoupled DNA replication and histone synthesis that occur after CX treatment induces a general modification of chromatin structure, and propose that this general disorganization of chromatin structure is responsible for a widespread activation of RNA polymerase II-mediated gene transcription.

INTRODUCTION

It is well established that chromatin plays a major role in the maintenance of a general repression of transcription [1]. Moreover, chromatin assembly that is tightly coupled to DNA replication is observed in eukaryotes [2,3], since it would be dangerous for a cell to synthesize naked DNA in the absence of chromatin assembly. This is because of the multiple roles of chromatin both in constraining inappropriate gene activity and in directing the appropriate packaging of DNA into chromosomes. Massive histone synthesis occurs during the S phase of the cell cycle, allowing replication-coupled chromatin assembly. Thus treatment of cells with an efficient inhibitor of protein synthesis, such as cycloheximide (CX), would be expected to have a dramatic effect on global chromatin structure during the S phase of the cell cycle. Indeed, the effect of CX on protein synthesis is very rapid, and occurs before its effect on DNA replication, which continues depending on the half-life of the replication machinery [4]. Therefore the formation of disturbed chromatin structure is expected in regions that have been replicated in the presence of CX [5,6]. Moreover, it has been shown that, in the presence of the protein synthesis inhibitor emetine, there is preferential inhibition of Okazaki fragment synthesis on retrograde arms of forks that produces ‘imbalanced’ DNA synthesis [7]. All of these events will have important consequences for the overall structure of chromatin.

The effect of CX on gene expression has long been of interest. The disappearance of regulators with short half-lives is the most popular hypothesis proposed to explain the effect of CX treat-

ment on gene expression (for a review, see [8]). In the present paper we consider a mechanism based on the ability of CX to uncouple DNA synthesis and chromatin assembly that may have widespread consequences in relieving chromatin-mediated general transcriptional repression. One important prediction of this model is an enhanced effect of CX treatment on S-phase cells compared with arrested cells. We undertook a series of experiments that showed an S-phase-dependent dramatic effect of CX on chromatin structure that is associated with general class 2 gene activation. We show, therefore, that chromatin is one of the most important elements mediating the response of RNA polymerase II (pol II)-transcribed genes to CX treatment. We believe that it is essential to consider this important indirect effect of CX on gene expression, since this inhibitor is widely used to examine the effect of translation on the regulation of specific gene expression. Moreover, although CX may have pleiotropic effects, its ability to induce an important alteration in chromatin structure may allow us to gain a better understanding of the relationship between chromatin structure and the control of transcriptional activity.

MATERIALS AND METHODS

Cell culture conditions

Murine erythroleukaemia (MEL) cells from clone G9, a subclone of F4NW0, were maintained in culture in minimum essential medium (Gibco) containing 10 % (v/v) fetal calf serum [9].

Abbreviations used: CX, cycloheximide; pol II, RNA polymerase II; CAT, chloramphenicol acetyltransferase; MEL, murine erythroleukaemia; TSA, trichostatin A.

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The clone 6 cell line, a rat embryonic fibroblast line transformed by Ras, was maintained in RPMI 1640 (Boehringer) supplemented with 5% (v/v) fetal calf serum and 4 mM glutamine, and grown in a humidified atmosphere of 95% air/5% CO₂. Growth was normally at 37 °C, but was shifted to 32 °C to induce growth arrest [10].

Northern blot analysis and the quantification of mRNA variation

RNAs were extracted from cells and analysed as described [9]. The quantification of variations in mRNA was performed by the densitometric measurement of mRNA signals. Values obtained were then normalized against signals obtained from both 28 S and 18 S rRNAs, as follows. The value corresponding to the signal obtained after the scan of a specific mRNA band was divided by each of the values obtained for the 18 S and 28 S rRNA bands, and the mean value of these two divisions obtained with non-treated cells (arrested or proliferating) was considered as the reference value (100%).

Cell transfection and primer extension

Clone 6 cells were transfected according to the protocol described in [11], using Lipofectin reagent (BRL) and 1 µg of a plasmid containing the chloramphenicol acetyltransferase (CAT) gene downstream of the histone H1^o promoter [11]. The H1^o-promoter-CAT construct used and the primer extension procedure were described elsewhere [11,12]. At 48 h after transfection, RNA was prepared from a cytoplasmic extract as described previously [13], and nuclei were used to measure the plasmid uptake, as reported by Peretti and Khochbin [14].

In vivo [³H]uridine pulse labelling and RNA fractionation

[³H]Uridine (50 µCi; 30 Ci/mmol) was added to MEL cell culture medium for 15 min before cell lysis. RNA was prepared as above and fractionated to poly (A)⁺ and poly (A)⁻ populations using an oligo(dT) column as described in [15]. RNAs were ethanol-precipitated, dried and counted for radioactivity.

In situ analysis of nuclear architecture

Clone 6 cells (cycling or arrested) after CX treatment were fixed with 2% paraformaldehyde (in PBS) and were stained with Hoeschst 33432. The preparation was observed under an epifluorescence microscope (Zeiss Axiophot) equipped with a 100 W mercury lamp using filter set no. 1 (BP 365; dichroic filter 395; LP 397) for Hoeschst fluorescence. Numeric image acquisitions were realized using a cooled CCD camera (C4880 Hamamatsu) mounted on the epifluorescence microscope. The nuclei were imaged using a 63×/1.25 normal aperture oil-immersion objective and an intermediate magnification (Optovar) of 1.25×. The size of the pixel was 0.0095 µm and the exposure time was between 2 and 6 s. In order to improve the quality of images blurred by 'out of focus' haze and glare, deconvolution was performed on grey level 16-bit images with software developed in our laboratory for the processing of fluorescent images using an algorithm introduced by Meinel [16]. A gaussian low-pass filter was also introduced as a noise reducer in order to obtain optimal image quality [17]. Image analysis was performed on a Silicon Graphics Workstation (Indy R4600) and PowerMac station for Image Editing (Apple).

RESULTS

Large-scale proliferation-dependent alteration of chromatin structure after CX treatment

In order to compare the cell-cycle-dependent effect of CX on chromatin structure in proliferating cells and in cells in the G₁ phase of the cell cycle, we used a temperature-sensitive model system consisting of cells transformed by a thermosensitive p53 mutant. At 37 °C, p53 is unable to control cell proliferation and cells show a high rate of division; however, at 32 °C, a p53-dependent arrest of cell proliferation occurs [10].

Proliferating cells at 37 °C or arrested cells kept at 32 °C were treated with CX for 3 h; they were then fixed and the global nuclear architecture was analysed *in situ*. After the staining of DNA by Hoeschst 33432 and the observation of samples under an appropriate microscope, images were recorded and processed as described in the Materials and methods section. Figure 1 shows a network of dense chromatin (highly fluorescent regions) in both arrested and proliferative cells (Figures 1a and 1c). After 3 h of CX treatment, a rearrangement of chromatin structure was observed both in arrested and in proliferative cells. However, the modification of the nuclear architecture was much more dramatic in proliferative cells. Indeed, fine and well defined regions of dense chromatin were still visible in arrested cells (Figure 1b), whereas these structures were more diffuse and spread all over the nuclei of proliferative cells (Figure 1d, arrows). Comparison of dense chromatin regions surrounding the nucleoli in arrested and proliferative cells illustrates this situation very well: nucleoli were detected in almost all nuclei in arrested cells (arrowheads in Figure 1b), while these structures were severely disrupted in the nuclei of proliferating cells (compare Figures 1b and 1d).

Figure 1 Modification of the nuclear architecture after CX treatment

Clone 6 cells either arrested in G₁ phase of the cell cycle (32 °C) or proliferating (37 °C) were treated with CX (20 µg/ml) for 3 h (T3) and the nuclear architecture was analysed after the staining of DNA with Hoeschst 33432. T0 indicates untreated cells. Arrowheads in (b) indicate structures associated with nucleoli, visible after 3 h of CX treatment. Arrows in (d) show the diffusion of dense chromatin structure in nuclei of CX-treated proliferative cells.

Figure 2 Rate of RNA synthesis during CX treatment

MEL cells were treated with CX (20 μ g/ml) for the indicated times. At 15 min before each time point, [3 H]uridine was introduced into the culture medium; 15 min later, cells were harvested and RNA was prepared. A fraction of the RNA was taken to measure the 3 H incorporation in total RNA, and the rest was loaded on to an oligo(dT) column. Flow-through material was dried and radioactivity was counted [poly(A)⁻ RNA curve]. The poly(A)⁺ RNA was eluted, dried and counted for radioactivity. A value of 100% represents the ratio of c.p.m./absorbance for untreated cells, and corresponds to the mean value of two measurements [52 606 c.p.m. for total RNA; 50 010 c.p.m. for poly(A)⁻ RNA and 2500 c.p.m. for poly(A)⁺ RNA]. In the case of poly(A)⁺ RNA, circles represent the values of each measurement expressed as a percentage of the control (0 h), and lines join the mean values. For total and poly(A)⁻ RNA, values are close to those obtained from total RNA, and are encompassed in a range represented by the bar.

General enhancement of pol II-mediated transcription after CX treatment

In order to show the effect of CX on general transcriptional activity, we performed a pulse-labelling experiment after different periods of treatment. MEL cells were chosen for this experiment, since we showed previously that, in an exponentially growing cell population, more than 70% of cells are in the S phase of the cell cycle [18]. MEL cells were treated for different times with CX and, 15 min before RNA preparation, [3 H]uridine was added to the culture medium. Purified RNA obtained from cells was loaded on to a poly(dT) column and the amount of 3 H incorporated into total RNA and into the poly(A)⁺-enriched and poly(A)⁻-enriched fractions was counted. There was a dramatic inhibition of class 1 and class 3 gene transcription (Figure 2), and this inhibition became more efficient as the length of treatment increased. However, during this period, class 2 gene transcription was not inhibited; moreover, some enhancement of transcription was observed.

S-phase-dependent stimulation of transcription after CX treatment

In this study we have tried to provide experimental evidence supporting the idea that uncoupled replication and chromatin assembly is responsible for the alteration of chromatin structure and the activation of class 2 gene transcription. This hypothesis predicts that the general activation of class 2 gene transcription after CX treatment described above would occur essentially in proliferating cells, rather than in G₁ arrested cells. We decided to use the clone 6 cell system, which enables synchronization of cells to be achieved by a shift in temperature, and two model genes, i.e. those encoding histone H1^o and histone H1. H1^o gene expression is developmentally regulated and the mRNA is present

Figure 3 S-phase-dependent activation of transcription of two pol II-transcribed genes

(A) Clone 6 cells kept at 37 °C (proliferating cells) or at 32 °C (G₁-arrested cells) were treated for the indicated times with CX (20 μ g/ml). RNA was purified and a Northern blot was obtained. The blot was probed with 32 P-labelled histone H1^o and H1 cDNA fragments. The curves (cytfluorimetric analysis of cells stained with Hoechst) represent the distribution, in the cell cycle, of cells kept at 37 °C and 32 °C respectively. (B) The amount of H1^o and H1 mRNA was quantified and normalized with respect to the amount of rRNA loaded, as described in the Materials and methods section. (C) Clone 6 cells kept at 32 °C were moved to 37 °C; one plate was treated with CX (20 μ g/ml) and cells from two plates, one untreated (—) and one CX-treated (CX), were lysed 3 h later (G₁ cells). The curve shows the state of cell proliferation at the moment of harvest. At 6 h after the temperature shift (start of S phase), CX was added to the culture medium; 3 h later, cells were lysed and RNA was prepared (the histogram shows the state of cell proliferation 9 h after the temperature shift). (D) A Northern blot corresponding to this experiment was probed and RNA variation was quantified as described above.

Figure 4 Co-operation between CX and an inhibitor of histone deacetylase, TSA, to induce gene expression in proliferative cells

RNA was prepared from proliferating (37 °C) or G₁-arrested (32 °C) clone 6 cells treated with CX (20 µg/ml), TSA (20 ng/ml) or both (CX + TSA) for 4 h. A Northern blot was obtained and probed with ³²P-labelled histone H1° and H1 cDNA fragments (A), and variations in the amount of RNA were quantified (B) as described in the legend to Figure 3.

in all phases of the cell cycle [19]. Histone H1 has a strictly S-phase-dependent expression [20]. We chose these two genes as a model because H1° gene expression is believed to be sensitive to the modification of chromatin structure essentially by histone acetylation [11,21], and histone H1 gene expression is cell-cycle-dependent.

Proliferating cells (at 37 °C) and arrested cells kept at 32 °C were treated with CX for different periods of time, and RNA was prepared and analysed by Northern blots. The blot was hybridized with histone H1 and H1° probes. Figure 3 shows that the expression of both genes was strongly induced after CX treatment, and this phenomenon occurred preferentially in proliferating cells (Figures 3A and 3B, 37 °C). The level of histone H1 mRNA is very low in G₁-arrested cells, and treatment of these cells with CX was incapable of inducing mRNA accumulation (Figures 3A and 3B, 32 °C). The treatment of arrested cells with CX for 2–4 h could induce H1° mRNA accumulation to some extent; however, this induction was 2–3-fold less than that observed in proliferative cells (Figure 3B).

In order to demonstrate the specific relationship between the induced expression of these genes and the S phase of the cell cycle, and also to exclude any effect of temperature on gene expression, we performed the following experiment. G₁ cells were obtained at 37 °C by replacing the media of cells kept at 32 °C (arrested cells) by fresh medium prewarmed at 37 °C, with and without CX. Plates were then placed in an incubator at 37 °C and cells were lysed 3 h later (Figure 3C, G₁ cells). Two other plates were kept at 37 °C for 6 h (when cells enter the S phase of the cell cycle; not shown); CX was then added to one plate and the cells were lysed 3 h later. Figures 3(C) and 3(D) show that CX

Figure 5 Effect of CX treatment on transcription from a transiently transfected promoter

Clone 6 cells were transfected with a plasmid containing a CAT gene under the control of H1° promoter [11,12]. After 48 h of transfection, cells were treated with CX for 2 h, and RNA was prepared from treated (CX) and untreated (—) cells. (A) A 15 µg portion of cytoplasmic RNA was subjected to a primer extension experiment using a primer located within the CAT coding sequence. The scheme on the right shows the position of the initiation site and the position of the primer used for the extension (the expected extension product is 105 bases long). Lane M contains size markers (bp). (B) Plasmid uptake was checked by Southern blot analysis of DNA extracted from the nuclei of transfected cells and hybridization with a plasmid probe.

treatment induced H1° and H1 gene expression very efficiently when the cells entered the S phase of the cell cycle, while cells in G₁ phase were less sensitive to the action of CX. This experiment also ruled out an effect of temperature in the reduced effect of CX on H1° gene expression observed in G₁ cells.

Co-operation between a histone deacetylase inhibitor and CX

Chromatin hyperacetylation following the treatment of cells with histone deacetylase inhibitors is also accompanied by a general modification of chromatin structure [22,23]. However, in contrast with the effects of CX, treatment of cells with histone deacetylase inhibitors affects only a small subset of genes [24]. The histone H1°-encoding gene used as a model in the present study is one of the genes that is induced specifically after histone hyperacetylation [11,21,25].

We attempted to demonstrate co-operation between the CX-mediated modification of chromatin structure and chromatin hyperacetylation in inducing specific gene expression. Proliferating clone 6 cells, or cells arrested at 32 °C, were treated for 4 h with CX plus trichostatin A (TSA), a specific inhibitor of histone deacetylase [26]. H1° was induced to a greater extent in cells treated with both CX and TSA than in cells treated with CX or TSA alone (Figures 4A and 4B, 37 °C, compare CX + TSA with CX or TSA alone). In arrested cells, TSA could induce H1° gene expression, but it did not co-operate with CX to induce H1°; moreover, CX reduced the extent of TSA-mediated H1° induction (Figures 4A and 4B, 32 °C). As shown previously, H1°

responds specifically to deacetylase inhibitors, and the treatment of cells with such drugs does not induce genes encoding other histones [11,21]. Here we confirm these findings and show that in, proliferative cells, although H1 is inducible by CX treatment, TSA alone had no effect on the expression of this gene, and it did not co-operate with CX to induce gene expression. In arrested cells, neither CX nor TSA treatment could induce H1 gene expression (Figure 4, H1).

Effect of CX treatment on the activity of a transfected promoter

In order to evaluate the effect of CX on a promoter not integrated into the bulk chromatin structure, we transfected proliferative clone 6 cells with a plasmid containing the promoter of the histone H1^o gene, possessing all the elements needed for efficient expression of the gene [11,12], and examined its activity, by primer extension, in control cells and in cells after 2 h of CX treatment. Figure 5 shows that CX treatment had only a modest effect on the transfected H1^o promoter activity, whereas, as shown before, it efficiently induced expression of the endogenous gene.

DISCUSSION

Rapid assembly of chromatin on newly replicated DNA is a critical stage in the maintenance of the correct structure and function of chromatin [27]. It is therefore reasonable to expect that even a slight uncoupling of chromatin assembly and replication would have a dramatic effect on the overall interphasic chromosome structure. Indeed, replication of DNA in the absence of histone synthesis would promote the local formation of non-canonical chromatin structures [5–7] that may disturb the higher-order chromatin structure such as the 30 nm fibre. Inhibition of protein synthesis uncouples replication and chromatin assembly [5–7] during a period that depends on the half-life of the replication machinery [4]. We sought, therefore, an approach to investigating the effect of the inhibition of protein synthesis on chromatin structure and function that is due essentially to the uncoupling of replication and chromatin assembly, by comparing the effects of CX on the chromatin structure and transcription of G₁-arrested cells and proliferative cells. This work was facilitated by the use of a special cell line transformed with Ras and a thermosensitive p53 [10]. At 37 °C, p53 is unable to control cell proliferation and cells show a transformed phenotype. However, at 32 °C, p53 takes over the control of the cell cycle and triggers the arrest of cells in G₁ phase. Therefore the temperature-dependent control of proliferation provides us with a model that allows comparison of the effects of CX treatment between proliferative and arrested cells, without the inconvenience of the traditional means of cell synchronization.

We first showed that, in contrast with arrested cells, the treatment of proliferating cells with CX triggered an important alteration to the structure of interphasic chromosomes. Moreover, [³H]uridine pulse-labelling showed that CX treatment enhanced overall class 2 gene transcription, whereas it efficiently inhibited transcription of class 1 and 3 genes, in agreement with previous observations [28]. The enhancement of the transcription of class 2 genes upon CX treatment is in agreement with the fact that, in general, class 2 genes are under a tight chromatin-dependent constraint, whereas class 1 genes are organized in a specialized chromatin structure that is adapted for a high rate of transcription [29,30]. Moreover, it was shown previously that the inhibition of protein synthesis results in a rapid depletion of transcription factors that are required for the initiation of the activity of RNA polymerases I and III [28,31].

We next used two pol II-transcribed genes as a model to show the S-phase-dependent effect of CX on transcription. One of these genes encodes histone H1, which, like core-histone-encoding genes, is expressed specifically in S phase of the cell cycle [20]; the other, the gene encoding histone H1^o, is expressed throughout the cell cycle [9]. Expression of both genes was enhanced after CX treatment in S phase cells. When G₁ phase cells were treated with CX, H1 mRNA did not accumulate, and H1^o mRNA accumulated only to a small extent. Previously we defined the molecular basis of the activation of the histone H1^o gene and showed not only that chromatin plays an important role in the control of gene expression, but also that hyperacetylation of histones activates transcription [11,12,21,25]. Therefore it is interesting to elucidate whether CX can co-operate with histone acetylation to superinduce the expression of the gene. Our data showed that, in proliferative cells, CX can enhance the effect of the histone deacetylase inhibitor TSA. In arrested cells, CX is incapable of enhancing the effect of TSA treatment; moreover, the TSA-mediated induction of H1^o is inhibited in these cells. It is now well established that histone deacetylases form part of different transcriptional regulatory complexes involved in silencing the transcription of specific genes [32,33]. Furthermore, a general histone deacetylase activity is also associated with the replication machinery, causing deacetylation of histones following chromatin assembly [34]. Treatment of cells with TSA promotes very rapidly the formation of hyperacetylated histones, such that almost all core histones in chromatin are hyperacetylated [21,26], and a drastic modification of chromatin structure is associated with this event [22,23]. H1^o expression is particularly sensitive to histone hyperacetylation, and is enhanced under these conditions [11,21,25,35]. This transcriptional stimulation occurs in G₀/G₁-arrested cells as well as in S phase cells, in agreement with the ability of the drug to hyperacetylate histones in both cell populations [21]. The fact that CX stimulates TSA-dependent H1^o expression only in proliferative cells, and not in G₁-arrested cells, suggests that it is essentially the modification of chromatin structure that enhances H1^o expression.

Finally, we showed that CX has only a modest effect on the activity of a transfected H1^o promoter. The transfected plasmid lacks a eukaryotic replication origin; therefore it does not replicate, and usually non-canonical chromatin structure is assembled on this kind of template [36]. These data suggest that the promoter needs to be present in the endogenous chromatin context in order to observe efficient transcriptional stimulation.

In this work we have shown a general effect of CX on chromatin structure and gene expression that is greatly enhanced in the S phase of the cell cycle. We performed a series of experiments suggesting that the S-phase-dependent effect is due essentially to uncoupled replication and chromatin assembly. However, it is clear that CX, as an inhibitor of translation, has an important effect on many different cellular events, and specific genes have been described to be activated or repressed by CX treatment, even in arrested cells, probably due to the disappearance of specific regulators of transcription after CX treatment [8]. However, the fact that transcription from the transfected promoter is not stimulated to the same extent as that of the endogenous gene indicates that (1) the correct endogenous chromatin context is essential in order to obtain induced expression after CX treatment, and (2) the possible disruption of other cellular events by CX does not interfere with the transcription of this gene. Therefore, one important aspect of this work is to highlight the general effect of CX on gene expression, specially in S phase, through the modification of chromatin structure. Secondly, the CX-dependent uncoupling of chromatin

assembly and replication is a powerful means for altering the structure of interphasic and mitotic chromosomes, and may provide important information for more detailed studies.

We are grateful to Dr. Jean Jacques Lawrence, the head of INSERM U309, for encouraging this work, to Marie-Paule Brocard for help in plasmid preparation, to Sandrine Curtet for help in tissue culture, and to Martina Creaven for English corrections.

REFERENCES

- 1 Wolffe, A. P. (1992) *Chromatin: Structure and Function*, Academic Press, London
- 2 Senshu, T., Fukada, M. and Ohashi, M. (1978) *J. Biochem. (Tokyo)* **84**, 985–988
- 3 Worcel, A., Han, S. and Wong, M. L. (1978) *Cell* **15**, 969–977
- 4 Schulte, D., Knippers, R., Dreier, T., Probst, G. and Probst, H. (1992) *FEBS Lett.* **299**, 149–154
- 5 Sogo, J. M., Stahl, H., Koller, T. and Knippers, R. (1986) *J. Mol. Biol.* **189**, 189–204
- 6 Kirov, N., Tsaneva, I., Yaneva, J. and Tsanev, R. (1987) *Biochem. Biophys. Res. Commun.* **145**, 1064–1070
- 7 Burhans, W. C., Vassilev, L. T., Wu, J., Sogo, J. M., Nallaseth, F. S. and DePamphilis, M. L. (1991) *EMBO J.* **10**, 4351–4360
- 8 Chesnokov, V. N. and Mertvetsov, N. P. (1991) *Biokhimiya* **55**, 1276–1278
- 9 Grunwald, D., Khochbin, S. and Lawrence, J. J. (1991) *Exp. Cell Res.* **194**, 174–179
- 10 Michalovitz, D., Halevy, O. and Oren, M. (1990) *Cell* **62**, 671–680
- 11 Khochbin, S. and Wolffe, A. P. (1993) *Gene* **128**, 173–180
- 12 Khochbin, S. and Lawrence, J. J. (1994) *Nucleic Acids Res.* **22**, 2887–2893
- 13 Khochbin, S. and Lawrence, J. J. (1989) *EMBO J.* **8**, 4107–4114
- 14 Peretti, M. and Khochbin, S. (1997) *J. Mol. Evol.* **44**, 128–134
- 15 Sambrook, J., Fritsch, E. F. and Maniatis, T. (1989) *Molecular Cloning: A Laboratory Manual*, 2nd edn., Cold Spring Harbor Laboratory Press, Cold Spring Harbor, NY
- 16 Meinel, E. S. (1986) *J. Opt. Soc. Am.* **3**, 787–799
- 17 Usson, Y. (1994) *Anal. Cell Pathol.* **6**, 201
- 18 Khochbin, S., Chabanas, A., Albert, P., Albert, J. and Lawrence, J. J. (1988) *Cytometry* **9**, 499–503
- 19 Khochbin, S. and Wolffe, A. P. (1994) *Eur. J. Biochem.* **225**, 501–510
- 20 Osley, M. A. (1991) *Annu. Rev. Biochem.* **60**, 827–861
- 21 Girardot, V., Yoshida, M., Beppu, T., Lawrence, J. J. and Khochbin, S. (1994) *Eur. J. Biochem.* **224**, 885–892
- 22 Perry, C. A. and Annunziato, A. T. (1989) *Nucleic Acids Res.* **17**, 4275–4291
- 23 Perry, C. A. and Annunziato, A. T. (1991) *Exp. Cell Res.* **196**, 337–345
- 24 Van Lint, C., Emiliani, S. and Verdine, E. (1996) *Gene Expression* **5**, 245–253
- 25 Almouzni, G., Khochbin, S., Dimitrov, S. and Wolffe, A. P. (1994) *Dev. Biol.* **165**, 654–669
- 26 Yoshida, M., Kijima, M., Akita, M. and Beppu, T. (1990) *J. Biol. Chem.* **265**, 17174–17179
- 27 Wolffe, A. P. (1994) *J. Cell Sci.* **99**, 201–206
- 28 Gokal, P. K., Cavanaugh, A. H. and Thompson, E. A. (1986) *J. Biol. Chem.* **261**, 2536–2541
- 29 Lucchini, R. and Sogo, J. (1992) *Mol. Cell. Biol.* **12**, 4288–4296
- 30 Mutskov, V., Russanova, V. R., Dimitrov, D. and Pashev, I. G. (1997) *J. Biol. Chem.* **271**, 11852–11857
- 31 Dieci, G., Duimio, L., Peracchia, G. and Ottonello, S. (1995) *J. Biol. Chem.* **270**, 13476–13482
- 32 Grunstein, M. (1997) *Nature (London)* **389**, 349–352
- 33 Struhl, K. (1998) *Genes Dev.* **12**, 599–606
- 34 Wade, P. A., Pruss, D. and Wolffe, A. P. (1997) *Trends Biochem. Sci.* **22**, 128–132
- 35 Seigneurin, D., Grunwald, D., Lawrence, J. J. and Khochbin, S. (1995) *Int. J. Dev. Biol.* **39**, 597–603
- 36 Jeong, S. J. and Stein, A. (1994) *Nucleic Acids Res.* **22**, 370–375