Role of Histone Modifications in Marking and Activating Genes through Mitosis*

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The global inhibition of transcription at the mitotic phase of the cell cycle occurs together with the general displacement of transcription factors from the mitotic chromatin. Nevertheless, the DNase- and potassium permanganate-hypersensitive sites are maintained on potentially active promoters during mitosis, helping to mark active genes at this stage of the cell cycle. Our study focuses on the role of histone acetylation and H3 (Lys-4) methylation in the maintenance of the competency of these active genes during mitosis. To this end we have analyzed histone modifications across the promoters and coding regions of constitutively active, inducible, and inactive genes in mitotic arrested cells. Our results show that basal histone modifications are maintained during mitosis at promoters and coding regions of the active and inducible RNA polymerase II-transcribed genes. In addition we have demonstrated that, together with H3 acetylation and H3 (Lys-4) methylation, H4 (Lys-12) acetylation at the coding regions contributes to the formation of a stable mark on active genes at this stage of the cell cycle. Finally, analysis of cyclin B1 gene activation during mitosis revealed that the former occurs with a strong increase of H3 (Lys-4) trimethylation but not H3 or H4 acetylation, suggesting that histone methyltransferases are active during this stage. These data demonstrate a critical role of histone acetylation and H3 (Lys-4) methylation during mitosis in marking and activating genes during the mitotic stage of the cell cycle.

In recent years it has become apparent that some histone modifications are involved in preserving the expression patterns through the cell division, contributing to the establishment of cellular memory. Histone methylation is believed to play an important role in maintaining the silenced heterochromatin (4, 9). More recently it has been shown in mammalian cells that di- and trimethylated H3 Lys-4 peaks correspond to the 5′-coding region, suggesting the implication of these modifications in the establishment and/or in the maintenance of potentially active states (8). In contrast to histone methylation, histone acetylation is not believed to play an important role in cellular memory, since it is a dynamic modification. However, the finding that bromodomains can bind preferentially to acetylated lysines (10) leaves open the possibility that histone acetylation could serve as an active mark during mitosis (4) as has already been proposed (11, 12). Nonetheless, the potential role of histone acetylation in maintaining the active configuration during the mitotic stage of the cell cycle is not completely understood. Within this context we have analyzed the contribution of acetylation and H3 Lys-4 methylation to marking and activating genes during mitosis.

EXPERIMENTAL PROCEDURES

Cell Culture and Synchronization; Mitotic Index Determination—HeLa S3 cells were grown in Dulbecco’s modified Eagle’s medium (Invitrogen) supplemented with 10% calf serum (Invitrogen). Cells were arrested in mitosis by treatment with 50 ng/ml nocodazole for 8 h. Mitotic cells were collected by selective detachment by manual shaking of the tissue culture flasks. The mitotic index was monitored by fluorescence-activated cell sorting analysis as follows. Cells were harvested after trypsinization, washed twice in PBS,2 and fixed in 70% ethanol for 2 h. Fixed and permeabilized cells were then stained with the fluorescent dye DAPI for 10 min and analyzed by flow cytometry with a BD Biosciences fluorescence-activated cell sorting scan according to the manufacturer’s instructions. The mitotic index was found to be 95–100%.

Indirect Immunofluorescence and Immunoblotting—Cells on cover slips were fixed in 4% p-formaldehyde in PBS for 30 min at room temperature and permeabilized with methanol for 10 min. After blocking with 3% bovine serum albumin in PBS, 0.1% Tween 20 for 1 h at room temperature, cover slips were incubated with a 1:200 anti-acetyl-H3 (Lys-9 and -14) antibody (Upstate Biotechnology), 1:200 anti-polyacetyl-H4 antibody (Upstate Biotechnology), 1:100 anti-phospho-H3 (Ser10) (Upstate Biotechnology), 1:200 anti-dimethyl-H3 (Lys-4) antibody (Upstate Biotechnology), 1:200 anti-trimethyl-H3 (Lys-4) antibody (Abcam), and 1:100 anti-acetyl-H4 (Lys-12) antibody (Upstate Biotechnology) in PBS, 3% bovine serum albumin for 2 h followed by incubation for 1 h with Cy3-conjugated goat anti-rabbit IgG, used at a 1:250 dilu-

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2 The abbreviations used are: PBS, phosphate-buffered saline; HAT, histone acetyltransferase; HDAC, histone deacetylase; HMT, histone methyltransferase; ChiP, chromatin immunoprecipitation; DAPI, 6′-diamidino-2-phenylindole; GAPDH, glyceraldehyde-3-phosphate dehydrogenase; IL, interleukin; ChiP, chromatin immunoprecipitation; H5, heat shock; Hsp70, Heat shock protein 70.
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**FIGURE 1.** Global histone acetylation and methylation in asynchronous and mitotic cells. A, fluorescence-activated cell sorting analysis of untreated (asynchronous) and nocodazole-treated (mitotic) HeLa S3 cells. 99% of mitotic cells were obtained after the treatment. B, Western blot analysis of asynchronous (A) and mitotic (M) HeLa S3 cells.

**Histones from asynchronous and mitotic cultures** were purified and analyzed by Western blot using antibodies that specifically recognize acetylated histone H3 at lysines 9 and 14 (Ac-H3), acetylated histone H4 at lysines 5, 8, 12, and 16 (Ac-H4), dimethylated H3 at lysine 4 (H3-k4-Dimet), trimethylated H3 at lysine 4 (H3-k4-Trimet), and phosphorylated H3 at serine 10 (H3-S10-P). C, immunocytological analysis of asynchronous and mitotic cells. p-Formaldehyde-fixed cells were stained with the antibodies indicated (B). Cells were co-stained with DAPI to reveal the DNA. D, relationship between chromatin condensation and H3 (Lys-4)-methylation levels. Immunocytological analysis of HeLa cells at various phases of mitosis (as indicated). Cells were stained with antibodies specific for either di- or trimethylated of histone H3 (Lys-4). E, cells were co-stained with DAPI to reveal the DNA. E, unequal subcellular distribution of HAT and HDAC activities during mitosis. Equal numbers of asynchronous and mitotic HeLa cells were extracted with IPH buffer containing 0.5% Triton X-100 on ice for 5, 10, or 15 min. The HAT and HDAC activities present in the resulting soluble and pellet fractions were determined by in vitro HAT and HDAC assays. Pellet/Soluble (P/S) ratios for each time and each activity (HAT and HDAC) are shown in the figure.

Histones from asynchronous and mitotic HeLa S3 cells were treated with 1% formaldehyde at room temperature for 15 min. The reaction was stopped by the addition of glycine to a final concentration 125 mM. Cells were washed once in ice-cold PBS, once with buffer I (0.25% Triton X-100, 10 mM EDTA, 50 mM Tris-HCl, pH 6.5) and with buffer II (200 mM NaCl, 10 mM EDTA, 50 mM Tris-HCl, 10 mM Hepes, pH 6.5) at 4 °C for 10 min. Subsequently, the pellet was resuspended in lysis buffer (1% SDS, 10 mM EDTA, 50 mM Tris, pH 8, 1 mg/ml protease inhibitors, 1 mM phenylmethylsulfonyl fluoride) and sonicated on ice until cross-linked chromatin was sheared to an average DNA fragment length of 0.2–0.5 kbp. After centrifugation (10–45 min 14,000 rpm), soluble cross-linked chromatin was diluted 1:10 in immunoprecipitation buffer (1% Triton X-100, 2 mM EDTA, 150 mM NaCl, 20 mM Tris, pH 8, and protease inhibitors), divided into aliquots, and stored at −80 °C. Chromatin preparations were precleared by incubation with a protein A-Sepharose solution (30 mg of preimmune serum (35 mg/ml), 2 mg of herring sperm DNA (1 mg/ml), 50 ml of protein A-Sepharose CL4B (Amersham Biosciences) 10% in Tris-EDTA buffer (50 mg/500 ml of Tris-EDTA) in immunoprecipitation buffer for 2 h at 4 °C under rotation. The protein A-Sepharose was removed by centrifugation; the pre-cleared chromatin was immunoprecipitated with the antibody O/N at 4 °C. The immunoprecipitates were washed with buffers TSE I (0.1% SDS, 1% Triton X-100, 2Mm EDTA, 10 mM Hepes, pH 6.5) and with TSE II (0.1% SDS, 1% Triton X-100, 2 mM EDTA, 20 mM Tris-HCl, pH 8, 500 mM NaCl), and buffer III (0.25 M LiCl, 1% Nonidet P-40, 1% deoxycholate, 1 mM EDTA, 10 mM Tris-HCl, pH 8) and three times with Tris-EDTA buffer. Washed pellets were eluted with 300 μl of a solution containing 1% SDS, 0.1 M NaHCO₃. Eluted
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**TABLE ONE**

| Characterization of antibodies recognizing modified histones H3 and H4 | Source or reference | Interphase | Mitosis |
|---|---|---|---|
| **Antibody** | **Antibody recognizes modifications** | **Antibody recognizes modifications** | **Antibody recognizes modifications** |
| Anti-acetyl-H3 | Lysines 9- and 14-acetylated H3 | Upstate Biotechnology 06-599 | + | + |
| Anti-acetyl-H4 | Lysines 5-, 8-, 12-, 16-acetylated H4 | Upstate Biotechnology 06-866 | + | +/- |
| Anti-phospho-H3 | Serine 10-phosphorylated H3 | Upstate Biotechnology 06-570 | - | + |
| Anti-dimethyl-H3 Lys-4 | Lysine 4-dimethylated H3 | Upstate Biotechnology 07-030 | + | - |
| Anti-trimethyl-H3 Lys-4 | Lysine 4-trimethylated H3 | Abcam ab8580 | + | + |

**RESULTS**

**Global Acetylation and H3 (Lys-4) Methylation at Interphase and Mitosis**—We first analyzed the levels of histone modification, which define active genes (H3 and H4 acetylation and H3 (Lys-4) di- and trimethylation) in global chromatin at interphase and mitosis. To this end HeLa S3 cells were synchronized in mitosis by treatment with nocodazole, which inhibits microtubule polymerization in a reverse manner. The mitotic index of the cell population after nocodazole treatment was measured by 1) fluorescence-activated cell sorting analysis and measured 90–95% (Fig. 1A) and 2) by microscopic examination of condensed chromosomes and nuclear envelope breakdown (data not shown). We then analyzed the acetylation of H3 (at Lys-9 and Lys-14 positions) and H4 polyacetylation (at Lys-5, -8, -12, and -16 positions) by immunoblotting and indirect immunostaining. Figs. 1, B and C, show that the level of H3 acetylation was maintained during mitosis (Figs. 1, B (2) and C (3 and 4)) H4 polyacetylation but decreased after the cells entered mitosis (Figs. 1, B (1) and C (1 and 2)), as was previously shown (18), and recovered by the end of the telophase (Ref. 18 and data not shown). We subsequently analyzed the H3 (Lys-4) di- and trimethylation levels of global chromatin at the interphase and during mitosis. Fig. 1, B (3 and 4) and C (5–8) show that in contrast to the global decrease of H4 polyacetylation, H3 (Lys-4) di- and trimethylation levels remained unchanged from the interphase to mitosis. Afterward, we analyzed the temporal relationship between the levels of di- and trimethylated histone H3 at Lys-4 and chromatin condensation. To this end we examined histone H3 (Lys-4) methylation states through mitosis. As shown in Fig. 1D, high levels of H3 methylation were observed on the chromosomes from the prophase to the telophase. This pattern differed from that of histone hyperacetylation; in this case, a concomitant decrease occurred as chromatin condensed into metaphase chromosomes and was re-established during late telophase (Ref. 18 and data not shown). These data suggest that histone methylation plays a different role than that of histone acetylation during mitosis. As expected, H3 (Ser-10) phosphorylation clearly peaked when the cells entered mitosis, remaining until late telophase (Fig. 1, B (5) and C (9 and 10)).

Unequal Subcellular Distribution of HAT and HDAC Activities during Mitosis—Mitotic global histone deacetylation has been postulated to occur by the displacement of HAT and HDAC enzymes from condensing chromosomes as cells undergo mitosis (18). However, if both activities were displaced from chromatin, the final histone acetylation/deacetylation equilibrium would be maintained. To get better insight into this problem, we have analyzed the subcellular distribution of HAT and HDAC activities in asynchronous and mitotic cells. To this end we extracted them with IPH buffer containing 0.5% Triton X-100 and incubated them in ice for different time periods to release loosely bound proteins (soluble fraction). The resulting pellet contained the tightly bound proteins, which included those strongly associated with fragments of the nuclear matrix (pellet). The distribution of HAT and HDAC activities in asynchronous and mitotic cells in soluble and pellet fractions was determined by in vitro HAT and HDAC assays, respectively. The level of HAT activity in the lysates changed from asynchrony to mitosis; indeed, during the latter, most of the HAT activity was present in the tightly bound fraction (Fig. 1E). However, this situation was completely different for HDAC activity, which was found mainly in the soluble fraction during mitosis (Fig. 1E). These findings suggest an unequal availability of HAT and HDAC enzymes, potentially explaining the global alteration in acetylation/deacetylation equilibrium, thereby leading to a global histone deacetylation during mitosis.

These data are consistent with previous results demonstrating a general loss of histone acetylation when cells undergo mitosis. This led us to analyze whether the promoter regions from active genes also lose acetyl-
Histone Acetylation and H3 (Lys-4) Methylation at Active and Inducible Promoters in Mitosis—To investigate the possible role of histone active modifications in the mitotic marking of active or inducible genes in living cells, we performed comparative ChIP analyses. Formaldehyde-cross-linked soluble chromatin was prepared from asynchronous and mitotic HeLa cells, normalized for DNA content, and subjected to immunoprecipitation with antibodies against acetylated H3 (Lys-9 and -14), polyacetylated H4 (Lys-5, -8, -12, and -16), di- and trimethylated H3 (Lys-4), and phosphorylated H3 (Ser-10) (TABLE ONE). Acetylation and methylation levels were determined by quantitative PCR analysis at the promoter regions of a constitutively active gene transcribed by RNA polymerase II GAPDH and an inactive RNA polymerase II-transcribed gene (IL-2).

We first compared the ChIP signals from asynchronous and mitotic cell populations. We found that the H3-acetylated basal signal at the GAPDH promoter was maintained during mitosis (Fig. 2A). The H4 acetylated basal signal slightly decreased relative to the asynchronous cells (Fig. 2A) but remained significantly higher than background levels obtained from the inactive gene promoter, IL-2 (Fig. 2B), during mitosis. H3 (Lys-4) methylation was subsequently analyzed at the GAPDH promoter in mitotic cells, finding that di- and trimethylated H3 (Lys-4) ChIP signals were similar to those from asynchronous cells (Fig. 2A). No methylated H3 (Lys-4) ChIP signals were found at the transcriptionally inactive promoter IL-2 (Fig. 2B). These data suggest that basal H3 and H4 acetylation as well as H3 (Lys-4) methylation marks are specifically maintained at the GAPDH promoter during mitosis (although a partial loss of H4 acetylation occurs).

After observing that activation marks are maintained during mitosis in the constitutively active RNA polymerase II-transcribed gene, we sought to analyze the promoter of an inducible gene, Hsp70. Fig. 2C shows that H3- and H4-acetylated ChIP signals in the Hsp70 promoter increased after heat shock (HS) stimulation of asynchronous cells as well as H3 (Ser-10) phosphorylation (Fig. 2C). H3 (Lys-4) methylation, however, did not significantly change after HS (Fig. 2C). These results suggest that both histone acetylation and H3 phosphorylation are involved in the rapid activation of the Hsp70 gene in asynchronous cells. In Drosophila, immunostaining studies have revealed that only H3 phosphorylation is involved in Hsp70 activation (19). However, mouse Hsp70 promoter has been postulated to require only histone acetylation to be activated after HS (20). Our ChIP results suggest that both modifications occur after HS activation in human cells.

Next, we compared the ChIP signals from asynchronous and mitotic cell populations. We found that the H3 acetylated basal signal was maintained during mitosis (Fig. 2D). The H4-acetylated basal signal decreased by 35–40%, relative to the asynchronous cells (Fig. 2D), but remained significantly higher than the background levels obtained from the inactive gene promoter (IL-2) during mitosis (Fig. 2B). After HS, the mitotic basal levels of acetylated H3 and H4 did not change (data not shown). Comparative ChIP analysis performed in parallel with the same numbers of asynchronous (+) and not heat-shocked cells (−) and analyzed by quantitative PCR using specific primers for the Hsp70 promoter. PCR products were resolved in agarose gels and quantified with QuantityOne software (Bio-Rad). The values on the y axis represent the amount of immunoprecipitated DNA as a percentage of the 1% input sample. Relative histone modification levels in mitotic (M) and asynchronous (A) cells were calculated from three independent experiments. A schematic representation of the GAPDH, IL-2, and Hsp70 promoters and coding regions as well as the position of the two primer pairs used for ChIP is shown at the top of each panel. A corresponds to HeLa cells heat-shocked at 43 °C for 30 min. Ab, antibody, t, trimethylation of Lys-4; d, dimethylation of Lys-4.
FIGURE 3. Acetylation and methylation of nucleosomes at coding regions in asynchronous (A) and mitotic (M) cells. ChIP analysis were performed as indicated in the legend of Fig. 2, and the immunoprecipitates were analyzed by quantitative PCR using specific primers for the GAPDH coding region (A), IL-2-coding region (B), and Hsp70-coding region (D). C corresponds to comparative ChIP analysis performed in parallel with the same numbers of asynchronous heat shocked cells at 43 °C for 30 min (+) and not heat-shocked cells (-) and analyzed by quantitative PCR using specific primers for the Hsp70-coding region. Ab, antibody; t, trimethylation of Lys-4; d, dimethylation of Lys-4.
shown). Finally, H3 (Lys-4) methylation was analyzed at the Hsp70 promoter in mitotic cells, finding that di- and trimethylated H3 (Lys-4) ChIP signals were similar to those from asynchronous cells (Fig. 2D).

These data confirm the above results; H3 and H4 acetylation marks are maintained during mitosis at the promoter regions of inducible and active genes. In the same manner, H3 (Lys-4) di- and trimethylation levels did not change during mitosis. As described above, the analysis of the H3, H4 acetylation, and H3 (Lys-4) methylation levels of the inactive RNA polymerase II-transcribed promoter IL-2 revealed no modifications either at synchrony or at mitosis; only H3 Ser-10 phosphorylation was detected after the cells underwent mitosis (Fig. 2B).

**Histone Acetylation and H3 (Lys-4) Methylation at Active and Inducible Genes during Mitosis**—It is possible that the maintenance of acetylation and methylation at active promoters serves to signal the reassembly of transcriptional machinery at the G1 or S phase; in such a case the promoter histone posttranslational modifications could play a pivotal role in marking active genes. We, therefore, analyzed whether the coding regions are also marked during mitosis by acetylation and methyla-

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**FIGURE 4.** H3 Lys-12 acetylation in asynchronous and mitotic cells. A, Western blot analysis of histones from asynchronous (A) and mitotic (M) HeLa S3 cells using antibodies that specifically recognize acetylated histone H4 at lysine 12 (Ac-H4-K12). B, Immunocytochemical analysis of asynchronous and mitotic cells with the antibody that specifically recognizes acetylated histone H4 (Lys-12) (Ac-H4-K12). Cells were stained with DAPI to reveal the DNA (blue). C, ChIPs analysis were performed as indicated in the legend of Fig. 2 using antibodies that specifically recognize acetylated histone H4 (Lys-12) (Ac-H4-K12). The immunoprecipitates were analyzed by quantitative PCR using specific primers for the promoters and coding regions of the indicated genes: GAPDH, IL-2, Hsp70, and cyclin B1.
tion. H3 acetylation ChIP signals of the GAPDH-coding region from mitotic cells were reduced by 15–20% compared with those from asynchronous cells during mitosis (Fig. 3A). On the other hand, H4 acetylation ChIP signals decreased by 40–50% in mitotic cells relative to asynchronous cells (Fig. 3A). We also analyzed the Hsp70-coding region using ChIPs. We initially observed that H3 and H4 acetylation as well as H3 phosphorylation occur both in the analyzed Hsp70-coding region (Fig. 3C) and in the promoter (Fig. 2C) after HS activation. We subsequently compared the ChIP signals from asynchronous and mitotic cell populations. We found that histone acetylation and H3 (Lys-4) tri-
methylation basal mitotic signals slightly decreased at the Hsp70-coding region relative to asynchronous cells (Fig. 3D). On the other hand, H3 (Lys-4) dimethylation levels did not change during mitosis (Fig. 3, A and D). As expected, neither histone acetylation nor methylation was detected at the IL-2-coding region (Fig. 3B). These results suggest that coding regions as well as promoters of active and potentially active RNA polymerase II-transcribed genes maintain the basal acetylation and H3 (Lys-4) methylation levels during the mitotic phase of the cell cycle. These histone posttranslational modifications may play a critical role in marking active gene during mitosis.

H4 Lys-12 Acetylation at Interphase and Mitosis—To identify potentially important sites for cellular memory within chromatin, Gottschling and co-workers (21) conducted a genetic screen in S. cerevisiae to isolate those mutant alleles of the H3 and H4 genes that set telomeric genes into a silenced state. They found that the level of acetylation at lysine 12 within the histone H4 tail functioned as a memory mark for propagating the expression state of a telomeric gene during mitosis. Based on this finding, we determined whether or not H4 (Lys-12) acetylation could affect the maintenance of transcriptional competency of euchromatic genes in mammalian cells. We studied the levels of acetylated H4 (Lys-12) on global chromatin at interphase and mitosis by immunoblotting and indirect immunostaining. Fig. 4. panels A and B, shows that the levels of acetylated H4 (Lys-12) are maintained during mitosis in bulk chromatin. We then analyzed acetyl-H4 (Lys-12) levels at the promoter and coding regions of the above-analyzed genes using ChIP assays; we have included the cyclin B1 gene since it is actively transcribed during mitosis (22). Our results demonstrate that H4 (Lys-12) acetylation is present at the coding regions of active and potentially active genes either in asynchronous or in mitotic cells (Fig. 4C). However, very weak or no ChIP signal was detected at the promoter regions of these active genes (Fig. 4C). Moreover, the promoter and coding region of the IL-2 gene were not reactive to anti-acetyl-H4 (Lys-12) antibody either in asynchronous or in mitotic cells (Fig. 4C). These results suggest that in histone H4, lysine 12 may serve as a memory mark in the nucleosome for the inheritable transmission of the active or inactive state in euchromatic genes, as has been suggested for telomeric genes (21).

Histone H3 (Lys-4) Methylation Couples with Transcription during Mitosis—It has been suggested that histone acetylation cannot occur during mitosis due to the displacement of HAT enzymes away from mitotic chromatin during the early prophase (18). We, therefore, decided to analyze whether the activation of mitotic-transcribed genes involves ongoing histone acetylation and/or methylation. To this end we analyzed the levels of acetylation at the cyclin B1 promoter during mitosis (when it was actively transcribed) using ChIP assays. The results in Fig. 5A reveal no changes in the H3 and H4 acetylation levels after gene activation during mitosis. However, the di- and trimethylated H3 (Lys-4) and phosphorylated H3 (Ser-10) signals clearly increased after gene activation (Fig. 5A). This suggests the existence of an ongoing HMT activity coupled with cyclin B1 transcription during mitosis. H3 (Ser-10) phosphorylation may be a necessary signal for mitotic entry, or it may be involved in cyclin B1 transcription activation, as has been suggested for other genes (19). Once we determined that active histone methylation at the promoter region may be involved in gene activation during mitosis, we analyzed whether it also occurs at the coding region of the cyclin B1 gene. Activation of this gene strongly correlates with an increase of trimethylated H3 (Lys-4) (Fig. 5B) but not of acetylated H3 and H4. In fact, the basal levels of acetylated H4 present in interphase decreased by 30–40% during mitosis (Fig. 5B), whereas acetylated H3 was not detectable in the analyzed region (Fig. 5B). These results suggest that HMTs are maintained as active enzymes during mitosis capable to methylate some mitotically active genes.

Based on this premise we analyzed the HMT activity in asynchronous and mitotic cells. As shown in TABLE TWO, the global HMT activity associated with mitotic and asynchronous cells was similar, suggesting the possibility that HMTs are maintained as active enzymes during mitosis.

**DISCUSSION**

Our current study demonstrates that although bulk chromatin is generally deacetylated during mitosis, acetylation and methylation marks of some active genes persist in the parental nucleosomes during mitosis. The general removal of acetyl groups from the modified lysines residues of core histones (23, 24) may be necessary for the compaction of chromatin, which involves neutralization of DNA negative charges (25). Several studies have shown that in the absence of histone H1, the acetylated nucleosome complexes remain in an extended conformation in contrast to the nonacetylated nucleosomes (26, 27). Furthermore, as a consequence of the linker histone binding required to allow high levels of chromatin compaction, inhibition of acetylation of oligonucleosomes occurs (28). On the other hand, the maintenance of some acetylated loci may be important in preventing some active genes from becoming silenced, as has been proposed (11, 12). Acetylation would serve as a signal for the propagation and maintenance of active chromatin states through cell division. How would histone modifications serve as an epigenetic mark during mitosis? According to the histone code hypothesis, different modifications are recognized by specific associated proteins responsible for reading the code (4, 29). Thus, permanent acetylation and methylation of promoter in active genes would serve to specifically bind certain effector proteins. Candidates for such interactions include (i) the Brd4 protein that contains two bromodomains and remains bound to mitotic chromatin in a bromo-dependent manner (30, 31) and (ii) TFIDD complex, which specifically interacts with acetylated histone H3 at lysines 9 and 14 (32). H3 (Lys-4) methylation could also provide a specific high affinity interaction surface for SNF2H binding (33) or for some chromo-domain-containing complexes (34). Interestingly, immunostaining analysis of the global H3 and H4 acetylation and H3 (Lys-4) methylation levels in mitotic cells revealed that residual mitotic acetylation and methylation correspond to discreet regions of the genomic chromatin, suggesting that previously active genes or genes that need to be rapidly reactivated after mitosis co-exist in the same chromatin domains.

Our results are consistent with Koussoudi and Talianidis (12) data for the human constitutively active hepatocyte nuclear factor 1 and 4 and albumin genes for H3 and H4 acetylation and H3 (Lys-4) methylation. Moreover, the present study expands the data for inactive but inducible (human Hsp70), inactive (IL-2), and mitotically active (cyclin B1) genes. In addition we have shown that H4 (Lys-12) acetylation at the coding regions in tandem with H3 acetylation and H3 (Lys-4) methylation contributes to the formation of a stable mark on active genes during mitosis. Finally, our results show that activation of cyclin B1 gene strongly cor-

| TABLE TWO |
|-------------------------------|------------------|
| **In vitro ratio of interphase/mitosis histone methyltransferase activity** |
| Total cell extract | Ratio of interphase to mitosis mean HMT activity (n = 3) |
| mg |  |
| 10 | 0.94 |
| 20 | 0.90 |
| 40 | 1.07 |
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relates with an increase of trimethylated H3 (Lys-4) but not H3 and H4 acetylation. Thus, these data provide the first evidence that HMTs may be maintained as active enzymes during this stage of the cell cycle. Therefore, the regulation of HMT enzymes during mitosis may differ from that of HAT/HDAC enzymes (18, 35, 36). This issue is currently under investigation.

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