Acetylation and Calcium-dependent Phosphorylation of Histone H3 in Nuclei from Butyrate-treated HeLa Cells*

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In HeLa nuclei, 1 μM Ca2+ stimulates 3-fold the phosphorylation of histone H3. Prior treatment of cells with Na butyrate increases the degree of H3 phosphorylation and reveals a correlation between the extents of H3 acetylation and phosphorylation. Acetylation of H3 increases its accessibility to the calcium-dependent kinase. Phosphorylation of H3 occurs at a serine residue located in the trypsin-sensitive region of the protein. Brief digestion of nuclei with DNase I preferentially releases the phosphorylated form of H3 from chromatin.

Protein phosphorylation is an important mechanism for regulating many biochemical processes within the cell (1, 2). Cyclic nucleotide-dependent phosphorylation has been established as an important component in such regulation; in addition, phosphorylation systems in many tissues contain cyclic nucleotide-independent kinases. For example, certain physiological effects of calcium may be mediated by calcium-dependent protein kinases (3).

We recently reported that HeLa nuclei contain a calcium-dependent protein kinase which selectively phosphorylates histone H3 (4). This activity is increased in nuclei from cells which have been grown in the presence of sodium butyrate, a treatment which increases the extent of histone acetylation (5–9). In view of the importance of the arginine-rich histones H3 and H4 in the compaction of nuclear DNA into chromatin (10–12) and because of the evidence implicating histone acetylation and phosphorylation in alterations in chromatin structure and function (13–15), we have characterized this nuclear H3 kinase activity in more detail.

Here, we describe experiments which analyze the H3 kinase activity and examine the relationship between butyrate treatment and the increase in H3 phosphorylation. Increased phosphate incorporation into H3 correlates with the degree of histone acetylation; acetylation apparently makes histone H3 a better substrate for the calcium-dependent kinase. We have also investigated the region of histone H3 which undergoes phosphorylation and the possible functional significance of the modification.

MATERIALS AND METHODS

Materials were obtained from the following sources: cell culture media, Gibco; plasticware, Falcon; [γ-32P]ATP (10–50 Ci/mmol), Amersham Corp.; trypsin and DNase I, Worthington; phosphoserine and phosphothreonine, Sigma; phosphotyrosine, a gift from Dr. Stanley Cohen, Vanderbilt University.

HeLa cells were grown as monolayers to near confluence in Dulbecco's modified Eagle's medium. Cells were washed and collected in ice-cold phosphate-buffered saline. Nuclei were prepared by Dounce homogenization in ice-cold buffer A (0.25 sucrose, 3 mM CaCl2, 1 mM Tris/Cl, pH 8) containing 1% Triton X-100. In the case of butyrate-treated cells, phosphate-buffered saline and buffer A also contained 5 mM Na butyrate. Nuclei were suspended (1–2 x 107 nuclei/ml) in 50 mM Tris/Cl, pH 7.5, 0.15 M NaCl, 5 mM MgCl2, 5 mM dithiothreitol, 5 mM Na butyrate, 1 μM ATP. Phosphorylation reactions were at 22 °C for 5 min. Unless indicated otherwise, free Ca2+ was maintained at 1 μM. Phosphorylation reactions were stopped by addition of EDTA to 10 mM and the nuclei were washed with 0.3 M. Histones were extracted with ice-cold 0.25 M HCl and precipitated with acetone, as previously described (4). Polyacrylamide gel electrophoresis and autoradiography were as previously described (4). Analysis of phosphoamino acids in H3 was performed as described previously (16).

RESULTS

Requirements for Enzyme Activity—Our standard conditions for studying histone H3 phosphorylation are based on the results of preliminary systematic studies in which we examined the time course of the reaction and its dependence upon the concentrations of ATP, cyclic nucleotides, NaCl, and free Ca2+. We incubated nuclei under various phosphorylating conditions, stopped the reaction with EDTA, washed the nuclei in 0.3 M NaCl to remove most nonhistone proteins, extracted the histones into HCl, fractionated them by polyacrylamide gel electrophoresis, and analyzed them for 32P by autoradiography (Ref. 4; see Figs. 3, 4, 5, and 7 of this report for examples of this type of analysis). These experiments revealed that, in nuclei from both control and butyrate-treated cells, the incorporation of 32P into H3 was maximal after a 5-min incubation with 1 μM ATP. Phosphorylation was maximal at an NaCl concentration of 0.15 M. Addition of either cyclic AMP or cyclic GMP (up to 10 μM) to the incubation had no effect on H3 phosphorylation. In contrast, EGTA markedly inhibited the reaction. These latter observations suggested that the H3 kinase was a calcium-dependent enzyme distinct from the cyclic nucleotide-dependent protein kinases. We therefore studied the calcium dependence of the phosphorylation reaction using a Ca2+-EGTA buffer (17). The effect of various concentrations of free Ca2+ on H3 phosphorylation is shown in Fig. 1. In nuclei from untreated cells, calcium produces a 2–3-fold increase in H3 phosphorylation with half-maximal stimulation occurring at a free Ca2+ concentration of about 0.1 μM. Butyrate treatment does not change the calcium sensitivity of the phosphorylation reaction (4). Thus, the H3 kinase is a cyclic nucleotide-independent enzyme, whose activity is stimulated by calcium at (presumably) physiological concentrations. We observed no calcium-dependent phospho-

1 The abbreviations used are: EGTA, ethylene glycol bis[β-aminoethyl ether]-N,N,N',N”-tetraacetic acid; SDS, sodium dodecyl sulfate; HMG, high mobility group.

* This research was supported by Grants CA 24680, MH 32752, and GM 30179 from the National Institutes of Health. The costs of publication of this article were defrayed in part by the payment of page charges. This article must therefore be hereby marked "advertisement" in accordance with 18 U.S.C. Section 1734 solely to indicate this fact.

† Recipient of a Faculty Research Award from The American Cancer Society.

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... of other histones under these conditions (4). However, we have found that the high mobility group protein HMG-17 is phosphorylated in a calcium-dependent manner (data not shown). We do not know whether the same kinase is involved in the phosphorylation of both histone H3 and HMG-17.

Effect of Butyrate—Butyrate inhibits histone deacetylase activity and therefore increases the extent of histone acetylation (6-9). In order to examine the relationship between H3 phosphorylation and acetylation, we asked whether the susceptibility of H3 to phosphorylation correlated with its degree of acetylation. We exposed cells to 0-50 mM Na butyrate for 16 h and prepared nuclei and incubated them with [γ-32P]ATP under standard phosphorylating conditions (see "Materials and Methods"). We extracted the histones and fractionated them by electrophoresis in polyacrylamide gels containing acetic acid and urea. We have observed that, in the presence of butyrate, changes in the extent of acetylation of both H3 and H4 occur in parallel, as assayed by the incorporation of [3H]acetate into the proteins (data not shown). Therefore, in the experiments described in Fig. 2, we estimated the extent of histone acetylation by quantitative densitometry of the H4 region of the Coomassie blue-stained gel because the acetylated forms of H4 are resolved better than the acetylated forms of H3 in this gel system (see, for example, Fig. 7). We measured the phosphorylation of H3 by quantitative densitometry of the autoradiogram. Fig. 2, left, shows that butyrate treatment causes a dose-dependent increase in H3 phosphorylation (lower section) which correlates with the increase in histone acetylation (upper section). Time course experiments (exposure to 10 mM Na butyrate for 0-24 h) also show a correlation between H3 phosphorylation and histone acetylation: phosphorylation and acetylation both exhibit half-maximal increases at approximately 3 h (Fig. 2, center). Furthermore, removal of Na butyrate from cells is followed rapidly by both a decrease in histone acetylation and a decrease in H3 phosphorylation (Fig. 2, right).

The studies with Na butyrate indicate that changes in H3 phosphorylation closely parallel changes in histone acetylation, suggesting a relationship between the two modifications. However, butyrate can have multiple effects upon the cell and could enhance the phosphorylation of H3 by affecting (a) the substrate, (b) the calcium-dependent kinase, or (c) a phosphatase that removes phosphate from H3. We tested the possibility that butyrate inhibits a phosphatase by measuring...
phospho-H3 phosphatase activity in nuclei from control and butyrate-treated cells. Nuclei were incubated with \(^{32}\text{P}\)ATP under standard phosphorylating conditions that produce \(^{32}\text{P}\)-labeled H3. The rate of H3 dephosphorylation was determined following the addition of EDTA to 10 mM, which inhibits further phosphorylation. At various times thereafter, aliquots were removed and analyzed for the amount of \(^{32}\text{P}\) remaining in H3, using polyacrylamide gel electrophoresis, autoradiography, and quantitative densitometry. The results shown in Table I indicate that the rate of loss of radioactive phosphate from H3 is similar in nuclei from both control and butyrate-treated cells. Thus, the enhanced phosphorylation of H3 in nuclei from butyrate-treated cells is not due to a decrease in phospho-H3 phosphatase activity.

**Table I**

**H3 phosphatase activity in nuclei from control and butyrate-treated cells**

| Time after addition of EDTA (min) | \(^{32}\text{P}\) remaining in H3 of control | % of control |
|----------------------------------|-------------------------------------------|-------------|
| 0                                | 100                                       | 100         |
| 2.5                              | 91                                        | 98          |
| 10                               | 89                                        | 98          |
| 40                               | 84                                        | 84          |

**Fig. 3. Mixing experiments.** Cells were grown for 16 h in the absence or presence of 5 mM Na butyrate. Nuclei were prepared, and aliquots of nuclei were made 0.45 m in NaCl and centrifuged (15,000 x g, 5 min). Supernatants were collected and adjusted to 0.15 m NaCl by addition of phosphorylation buffer containing 30 NaCl. Aliquots of supernatant were then combined with the salt-washed nuclear pellets, and phosphorylation reactions were performed under standard conditions (see “Materials and Methods”). The reaction was stopped by addition of EDTA to 10 mM. Nuclei were washed with 0.3 m NaCl and extracted with 0.25 m HCl. Phosphorylation of H3 was determined by electrophoresis in SDS-polyacrylamide gels, followed by autoradiography and quantitative densitometry.

**Fig. 4. Phosphorylation of acid-extracted histones by H3 kinase activity extracted from HeLa cell nuclei.** Histone substrates were prepared from salt-washed (0.3 m NaCl) nuclei from untreated or butyrate-treated (5 mM, 24 h) cells by extraction into 0.4 N HCl, followed by precipitation with 3 volumes of ethanol. H3 kinase activity was extracted from nuclei of untreated cells with 0.45 m NaCl. The salt extract was adjusted to a final concentration of 0.15 m NaCl using phosphorylation buffer containing no NaCl. Acid-extracted histone was incubated with the salt extract in the absence or presence of EDTA (5 mM) under standard phosphorylating conditions (see “Materials and Methods”). The reaction was stopped by the addition of EDTA to 10 mM; histones were extracted with 0.25 m HCl, precipitated with acetone, and analyzed by SDS-polyacrylamide gel electrophoresis and autoradiography. **Left section,** stained gel; **right section,** autoradiogram. **Lane A,** nuclei from untreated cells, unwashed; **Lane B,** nuclei from untreated cells, unwashed; **Lane D,** nuclei from butyrate-treated cells, washed with 0.45 m NaCl; **Lane E,** salt-washed nuclei from untreated cells, combined with salt extract from untreated cells; **Lane F,** salt-washed nuclei from untreated cells, combined with salt extract from butyrate-treated cells; **Lane G,** salt-washed nuclei from butyrate-treated cells, combined with salt extract from untreated cells; **Lane H,** salt-washed nuclei from butyrate-treated cells, combined with salt extract from untreated cells. Electrophoresis was from top to bottom. The arrow indicates the position of H3.
gel electrophoresis and autoradiography. As shown in Fig. 4 (Lanes A and B), the kinase preparation phosphorylates acid-extracted histones from butyrate-treated cells in a calcium-dependent manner. This, as well as the specificity for histone H3, strongly suggests that we are measuring the appropriate H3 kinase activity in this experiment. We observed a similar degree of calcium-dependent H3 phosphorylation when the reaction was carried out using acid-extracted histones from control cells (Fig. 4, Lanes C and D). Thus, histones purified from butyrate-treated cells do not exhibit an enhanced susceptibility to phosphorylation, compared to that of histones purified from control cells. Control experiments using histones without kinase (Lanes E and F) or kinase without histones (Lane G) revealed no phosphorylation of H3. Thus, our observations suggest that acetylation, per se, does not enhance the susceptibility of H3 to phosphorylation; therefore, the increased H3 phosphorylation in butyrate-treated nuclei presumably occurs secondary to an alteration in chromatin conformation resulting from histone acetylation.

Phosphorylated Site in H3—The amino acid residues in H3 which undergo phosphorylation or acetylation are located in the NH2-terminal region of the protein (12, 13), a region which is sensitive to digestion by trypsin (18-20). Here, we have used trypsin to show that the nuclear calcium-dependent kinase phosphorylates H3 in its NH2-terminal region. Nuclei from untreated cells were phosphorylated under standard conditions; the reaction was stopped with EDTA, and the nuclei were digested with trypsin to selectively remove the NH2-terminal region of the histones. The proteins in the digest were analyzed by SDS-polyacrylamide gel electrophoresis, followed by autoradiography. The stained gel in Fig. 5 shows that trypsin digests the core histones in control nuclei in a pattern similar to those reported previously, indicating that trypsin has selectively removed the NH2-terminal region of the proteins. Histone H3 (upper arrow) is digested to a more trypsin-resistant smaller fragment (lower arrow) which migrates just above histone H4. The autoradiogram indicates that, as digestion proceeds, there is a decrease in 32P-labeled H3 and there is no 32P associated with the smaller fragment of H3, containing the central and carboxyl-terminal regions of the protein. We observed identical results in analogous experiments using nuclei from butyrate-treated cells (data not shown). Thus, our results indicate that, in both control and butyrate-treated cells, the phosphorylation of H3 occurs in the trypsin-sensitive NH2-terminal region of the histone.

Dixon et al. (13) have reported that H3 undergoes phosphorylation at serine; in addition, Shoemaker and Chalkley (21, 22) have described an H3-specific kinase from calf thymus nuclei which phosphorylates the protein at threonine. Because of the possibility that butyrate treatment might alter the pattern of amino acid phosphorylation in H3, we determined the amino acid(s) in H3 which is phosphorylated in both control and butyrate-treated nuclei. We phosphorylated nuclei, fractionated the histones by SDS-polyacrylamide gel electrophoresis, and eluted H3 from the gel. We hydrolyzed the eluate and analyzed it for phosphoamino acids using paper electrophoresis and a pyridine/acetate acid buffer system (16). The results (Fig. 6) indicate that, in both control and butyrate-treated cells, at least 95% of the radioactive phosphate co-migrates with phosphoserine; in each case, a trace amount of radioactivity co-migrates with phosphothreonine, while no detectable radioactivity co-migrates with phosphotyrosine. Thus, the calcium-dependent kinase phosphorylates serine, and butyrate treatment produces no detectable change in the pattern of amino acid phosphorylation in H3.

DNase I Digestions—Both acetylation and phosphorylation of the histones are associated with changes in chromatin structure and function (10, 13-15). We have taken advantage of the observation that DNase I preferentially degrades chromatin DNA which is in an "active" configuration (23-27) to ask whether phosphorylated H3 is associated with such regions of the nucleoprotein. Nuclei from butyrate-treated cells were phosphorylated and subsequently were briefly digested with DNase I. The nuclear proteins released from chromatin

**Fig. 5. Trypsin digestion of phosphorylated nuclei.** Nuclei were prepared from untreated cells and phosphorylated under standard conditions (see "Materials and Methods"). The reaction was stopped by addition of EDTA to 10 mM, and the mixture was divided into six aliquots. Each aliquot was made 0.45 M in NaCl and centrifuged (15,000 x g, 5 min). The salt wash was repeated, and the pellets were resuspended in 5 mM CaCl2, 100 mM Tris/Cl, pH 7.5. Trypsin was added to a final concentration of 50 μg/ml, and the nuclei were digested at 37 °C. At the indicated times, the reaction was stopped by the addition of HCl to a final concentration of 0.25 N. Acid-extracted proteins were precipitated with acetone and analyzed by SDS-polyacrylamide gel electrophoresis and autoradiography. Left section, stained gel; right section, autoradiogram. Lane A, no trypsin; Lane B, 0-min digestion; Lane C, 5-min digestion; Lane D, 10-min digestion; Lane E, 20-min digestion; Lane F, 40-min digestion. Electrophoresis was from top to bottom. The upper arrow indicates the position of H3. The lower arrow indicates the position of the trypsin-digested fragment.

**Fig. 6. Electrophoresis and autoradiography of phosphoamino acids in H3 from untreated and butyrate-treated cells.** Cells were grown in the absence or presence of Na butyrate (5 mM, 24 h). Nuclei were phosphorylated under standard conditions (see "Materials and Methods"). Histones were extracted and fractionated by SDS-polyacrylamide gel electrophoresis. The gel was stained briefly (5 min) with Coomassie blue. The region containing H3 was cut from the gel, and H3 was eluted from the gel by incubation overnight in 0.5% SDS at 37 °C. The eluate was lyophilized and resuspended in H2O, and an aliquot was assayed for radioactivity by scintillation counting. Carrier histone was added to samples containing approximately equal amounts of radioactivity, and the samples were precipitated overnight at 0 °C in 20% trichloroacetic acid. Precipitates were collected by centrifugation, suspended in 6 N HCl, and incubated at 100 °C for 4 h. Acid was removed by evaporation, and the hydrolysis products were dissolved in H2O and subject to paper electrophoresis together with phosphoamino acid standards at room temperature at pH 3.5 using a pyridine/acetate acid buffer system (19). Electrophoresis was for 3 h at 800 V. The paper was dried and sprayed with ninhydrin to identify the positions of the standards, and subject to autoradiography. Upper lane, H3 from untreated cells; lower lane, H3 from butyrate-treated cells. The positions of the standards are as follows: A, phosphoserine; B, phosphothreonine; C, phosphotyrosine. O indicates the origin.
as a result of the nuclease digestion were analyzed by acid/urea/polyacrylamide gel electrophoresis and autoradiography and were compared with those proteins which remained associated with the nucleoprotein complex. Fig. 7 shows that no histone is released from chromatin in the absence of nuclease digestion (Lanes A and B). As digestion proceeds, an increasing amount of histone is released, roughly corresponding to the amount of DNA which is digested to acid-soluble form (i.e. approximately 10% of the total histone is released when 10% of the DNA is digested to acid solubility). To facilitate the analysis of the nuclease-digested samples, we electrophoresed approximately equal amounts of histone protein from each sample, as measured by staining with Coomassie blue. Examination of the stained gel reveals that the forms of H4 released by brief digestion with DNase I are, on the average, significantly more highly acetylated than the forms of H4 remaining in the pellet (compare Lanes C and E with Lanes D and F). Presumably, therefore, DNase I also releases the more highly acetylated forms of H3. Examination of the autoradiogram reveals that DNase I preferentially releases the phosphorylated form of H3 (as well as many nonhistone phosphoproteins) from chromatin. Densitometric scans of the autoradiograms indicate that following digestion, the specific activity of H3 which is released during digestion is about 3-fold that of the H3 which remains bound in the nucleoprotein complex (data not shown). We observed similar preferential solubilization of phosphorylated H3 in analogous experiments using nuclei from control cells (data not shown). Thus, these experiments suggest that the phosphorylated form of H3 (perhaps because it is also more highly acetylated) is associated with DNase I-sensitive regions in chromatin.

**Fig. 7. DNase I digestion of phosphorylated nuclei.** Nuclei were prepared from butyrate-treated cells (5 mM, 24 h) and phosphorylated under standard conditions (see “Materials and Methods”). The reaction was stopped by the addition of nonradioactive ATP to a final concentration of 20 μM. DNase I was added to a final concentration of 50 units/ml, and nuclei were digested at 22 °C. Aliquots were removed at the indicated times, and EDTA was added to a final concentration of 10 mM. A portion of the aliquot was removed for determination of perchloric acid-soluble material absorbing at 260 nm. The remaining portion was centrifuged (15,000 × g, 5 min), and both the supernatant and the pellet were then extracted with 0.25 M HCl (0°C, 60 min). Acid-extractable proteins were precipitated overnight with acetone at −20 °C and analyzed by electrophoresis in polyacrylamide gels containing acetic acid and urea, followed by autoradiography. In order to facilitate the analysis, lanes C and E (supernatants) were loaded with a higher fraction of the total protein than the corresponding pellets, lanes D and F (see text). Left section, stained gel, right section, autoradiogram. Lanes A (supernatant) and B (pellet), undigested nuclei; Lanes C (supernatant) and D (pellet), 1-min digestion, 4% perchloric acid-soluble A\textsubscript{NC}, Lanes E (supernatant) and F (pellet), 3-min digestion, 10% perchloric acid-soluble A\textsubscript{NC}. Electrophoresis was from top to bottom. Brackets indicate the positions of H3 and H4.

**DISCUSSION**

We have extended our studies of the calcium-dependent phosphorylation of histone H3 in HeLa cell nuclei and its enhancement by butyrate treatment. Our experiments reveal a correlation between increased histone acetylation and increased H3 phosphorylation. Butyrate enhances H3 phosphorylation by increasing the susceptibility of the histone substrate to phosphorylation by the calcium-dependent kinase. This enhanced susceptibility to phosphorylation probably reflects an increased accessibility of H3 secondary to an altered chromatin conformation in butyrate-treated cells. Phosphorylation occurs at a serine residue(s) located in the trypsin-sensitive NH\textsubscript{2}-terminal region of the histone. The phosphorylated H3 is preferentially released by DNase I digestion; this may mean that it is associated with regions of the genome which are potentially transcribable. Alternatively, this finding may primarily reflect an alteration in the solubility properties of acetylated chromatin.

Cells exposed to Na butyrate exhibit a variety of structural and functional changes (29). Na butyrate inhibits histone deacetylase activity, leading to the accumulation of hyperacetylated core histones, particularly H3 and H4 (6–9, 30). In duck erythrocytes, Na butyrate increases the degree of acetylation of high mobility group proteins HMG-14 and HMG-17 (31). In HeLa cells, Na butyrate enhances the phosphorylation of HMG-14 and HMG-17 (32). In contrast, in both mouse Ehrlich ascites cells and human colon carcinoma (HT-29) cells, treatment with Na butyrate inhibits the phosphorylation of these HMG proteins (33). Several reports indicate that Na butyrate treatment produces alterations in the pattern of chromatin transcription (34–41). HeLa cells which have been exposed to Na butyrate exhibit several changes in the pattern of histone phosphorylation in addition to those which we have described here. Butyrate inhibits the phosphorylation of histones H1 and H2A. These effects of butyrate are time-dependent, concentration-dependent, and reversible; they cannot be accounted for on the basis of changes in either kinase activity or phosphatase activity (42). Thus, Boffa et al. (42) suggest that in butyrate-treated cells, the inhibition of H1 and H2A phosphorylation may reflect alterations in the accessibility of the histone substrates, which occur secondary to a change(s) in chromatin structure elicited by butyrate.

The change(s) in chromatin structure, which occurs as a result of butyrate treatment, is also evident in the increased accessibility of chromatin DNA to DNase I (43–46). At moderate ionic strength (0.15–0.20 M NaCl) similar to the conditions we have used here, the nuclease preferentially digests hyperacetylated chromatin to Mg\textsuperscript{2+}-soluble form (47). In contrast, the selectivity of DNase I for hyperacetylated chromatin is greatly decreased when purified core particles are used as substrate (45). These observations suggest that acetylation of the histones can reduce internucleosomal interactions in chromatin, producing an alteration(s) in higher order chromatin structure and the increased accessibility of these regions to DNase I. This interpretation is consistent with our previous studies of trypsin-digested core particles, which implied that some of the NH\textsubscript{2}-terminal regions of the core histones might contribute to internucleosomal interactions and higher order chromatin structure (48). The NH\textsubscript{2}-terminal region of histone H3 seems to be particularly accessible in chromatin, in view of its relative susceptibility to trypsin digestion, to modification by histone acetylase(s) and deacetylase(s), and to phosphorylation by a calcium-dependent kinase, as we have shown here. Thus, our findings, together with those of other workers, are consistent with the idea that acetylation and/or phosphorylation of H3 may contribute to alterations in higher order chromatin structure.
The H3 kinase activity described here differs from that of the enzyme purified from calf thymus (21, 22). Both enzymes are selective for histone H3 and phosphorylate an amino acid in the NH2-terminal portion of the protein; however, the calf thymus enzyme phosphorylates a threonine residue (21, 22), whereas the enzyme described here phosphorylates a serine residue. Both enzymes are cyclic nucleotide-independent kinases. Our results indicate that the HeLa enzyme is stimulated by Ca2+; no such effect has been reported for the calf thymus enzyme. These differences may reflect species or tissue differences in cyclic nucleotide-independent H3-specific kinases. In addition, Taylor (49) has reported that, in calf thymus chromatin, H3 can be phosphorylated in vitro at serine residue 10 by a cyclic AMP-dependent protein kinase.

The activity described here is selective for histone H3 whether the protein is present as part of a nucleoprotein complex or in a mixture of purified acid-extracted histones. The kinase phosphorylates acid-extracted H3 in a calcium-independent manner; however, purified acetylated forms of the substrate do not exhibit enhanced susceptibility to phosphorylation by the enzyme. The enzyme is present in Triton-washed nuclei and is extracted from nuclei only after the NaCl concentration is raised to 0.45 M. Thus, it is presumably associated with chromatin in the intact cell. Such kinases, in purified form, may be useful for labeling H3 to high specific activity for use in experiments involving the fractionation of chromatin components or reconstitution of the nucleoprotein.

Calcium-dependent protein kinases are thought to play an important role in mediating the response of the cell to changes in its external environment, such as stimulation by hormones, neurotransmitters, drugs, or mitogens (3). Our experiments reveal the presence of a calcium-dependent nuclear enzyme capable of phosphorylating histone H3, a chromosomal protein thought to be of fundamental importance in chromatin structure and function. Our observations, therefore, suggest a potential biochemical mechanism whereby stimuli at the cell surface can produce molecular changes at the level of chromatin.

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