The in Vitro Phosphorylation of Chromatin by the Catalytic Subunit of cAMP-dependent Protein Kinase*

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When a mixture of DNA-free core histones (H) from calf thymus is phosphorylated by the catalytic subunit of cAMP-dependent protein kinase, phosphate is incorporated primarily into H2B and, to a lesser extent, into H5 and H4. In contrast, when the phosphorylation of the DNA-free histones is compared to the phosphorylation of histones in long chromatin or in nucleosome core, only one of the core histones, H3, is phosphorylated.

The site of modification of H3 has been identified as serine 10, which is located in the highly charged basic NH2-terminal region of the molecule. The other sites of phosphorylation in H2B and H4 are completely masked when the histones are complexed with DNA. It is only when the nucleosome core structure is perturbed that these additional sites become accessible to the kinase.

If long chromatin which contains H1 is phosphorylated, histone 1 is also phosphorylated by the catalytic subunit at serine 38. However, the addition of excess H1 to H1-depleted long chromatin inhibits the phosphorylation of H3.

In addition to the histones, the high mobility group (HMG) protein, HMG 14, was also found to be a good substrate for the kinase. Phosphorylation of HMG 17 in comparison was much less.

cAMP-dependent protein kinases have been shown to play a role in the regulation of a wide variety of enzymes and proteins (1, 2). The enzyme was first demonstrated to play a regulatory role in the metabolism of glycogen in the liver where it was eventually shown that both glycogen synthase and phosphorylase kinase were phosphorylated by the CAMP-dependent kinase and that the activity of both enzymes was modulated by that phosphorylation (3, 4). Since that time numerous enzymes and proteins have been shown to be physiological substrates for this kinase (1). By characterizing the phosphorylation sites in these protein substrates (5, 6), as well as by utilizing synthetic peptide substrates (7, 8), it has been established that despite its wide range of protein substrates the kinase does in fact have a clear sequence specificity. As a general rule, all of the sites that are phosphorylated in native proteins by this kinase have the following sequence, Arg-Arg-X-Ser(P)-X, where the site of phosphorylation is preceded by two basic residues, usually arginine. The actual site of phosphorylation in native proteins is usually the hydroxyl group of a serine residue although in synthetic peptides threonine and even hydroxyproline will serve as phosphate acceptors (9). There is usually a small hydrophobic residue on either side of the serine although this is not mandatory since in some instances the serine is adjacent to the basic residues (5-9). In native proteins no more than two residues have been found between the basic amino acid and the phosphorylatable serine.

The conventional and most widely accepted assay for the catalytic subunit of cAMP-dependent protein kinase utilizes a commercial mixture of calf thymus histones as substrate (10). Histones are very basic proteins with multiple sites of phosphorylation. This, in addition to the fact that they are commercially available in large quantities, makes them a very convenient substrate rather than the more physiological substrates such as phosphorylase kinase, pyruvate kinase, or glycogen synthase.

On the other hand, utilization of histones as a substrate for kinase is clearly not physiological in terms of functionally significant phosphorylations of histones since histones almost never exist in their free form within the cell. Under physiological conditions, histones are complexed with DNA almost immediately following their synthesis (11). For this reason and also in order to determine the effect of DNA on the accessibility of histone sites to phosphorylation, the specificity of histone phosphorylation was determined in chromatin where histones exist as a complex with DNA.

EXPERIMENTAL PROCEDURES

Proteins Used—The catalytic subunit of cAMP-dependent protein kinase was purified from porcine cardiac muscle as described previously (12). Nuclei, long chromatin, and nucleosome core were prepared from beef kidney as described previously by Lutter (13). Tetramer containing equivalent amounts of histones 3 and 4 was prepared from beef kidney using the hydroxyapatite method described by Simon and Felsenfeld (14). Histone mixtures were obtained from Sigma (type IIA) or Boehringer Mannheim. Purified H3 was purified by acid extraction from calf thymus according to Johns (15). H1, HMG 14, and HMG 17 were purified from beef kidney using a modified ion exchange method (16).

Assay and Phosphorylation Conditions—Kinase activity was assayed as described previously (17) at 37 °C in 50 mM Tris (pH 7.0) using [γ-32P]ATP (Amersham Corp.) > 2000 Ci/mmol. Unless otherwise indicated, all phosphorylations were carried out in this buffer at 37 °C using 0.6 mM ATP, 20 mM MgCl2, and 20 μg of catalytic subunit per ml. Aliquots were removed and added to 50 μl of ice-cold stopping solution (5% bovine serum albumin, 3.7% ethylenediaminetetraacetic acid, sodium salt). The samples were then spotted on Whatman No. 3MM filter disks and washed 3 times in 10% trichloroacetic acid.

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The abbreviations used are: H, histone; HMG, high mobility group; SDS, sodium dodecyl sulfate; Me, methyl; Ac, acetyl; TPCK, L-1-tosylamido-2-phenylethyl chloromethyl ketone; DITC-glass, the phenylethylisothiocyanate derivative of β-amino propyl glass.

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acid with stirring. After a final wash in 95% ethanol, the filter disks were air-dried and counted in 5 ml of Aquasol scintillation fluid (New England Nuclear).

When incorporation into individual histones was determined, aliquots were removed and added directly to gel electrophoresis stopping buffer (final concentration, 25 mM Tris, 0.2% SDS, 10% glycerol, 2% 2-mercaptoethanol). Samples were then electrophoresed as described previously (18) using 18% acrylamide gels with a thickness of 1.5 mm. A stacking gel of 4.6% acrylamide and 0.37% bisacrylamide with a thickness of 7.5 cm was used. Samples were taken up in 0.2% SDS, 10% glycerol, 25 mM Tris, 2% 2-mercaptoethanol, and placed in a boiling water bath for 5 min. Electrophoresis was carried out at 40 mA until the sample was into the stacking gel and then increased to 60 mA for the duration of the electrophoresis. Acidurea gelatin gels were run according to Allfrey et al. (19), using a stacking gel of 7.5% acrylamide and 0.125% methylenebisacrylamide.

Peptide Isolation and Mapping—The lyophilized protein was digested with a 1:50 (w/w) ratio of TPCK-trypsin (Worthington) for 15 h at 37 °C in 50 mM NH4HCO3 (pH 8.1). The sample was then applied directly to a Sephadex G-25 column (2150 cm) equilibrated in 0.1% NH4HCO3. Peaks containing radioactive peptides were pooled, lyophilized, and resuspended in a minimum volume of water. The sample was then applied to a Bondasil C (Waters Associates, Inc.) column. The column was eluted with a 0-50% linear gradient of buffer B where buffer A is 0.1% trifluoroacetic acid and buffer B is acetonitrile. The elution was done at a flow rate of 1 ml/min for 30 min using an Altex dual pump (model 110A) system.

In addition to isolating peptides from purified individual histones, peptide maps and sequence analyses were carried out on histones isolated following polyacrylamide gel electrophoresis. The protein to be mapped was subjected to polyacrylamide gel electrophoresis as described above. Following electrophoresis, the gel was stained rapidly (less than 30 min) with 0.1% Coomassie blue (0.1% Coomassie brilliant blue R250 (Gurr) in MeOH:H2O:HAc (5:6:4)). After destaining in 7% HA:5% MeOH, the gels were dried and the individual protein bands cut out and counted in 5 ml of Aquasol scintillation fluid (New England Nuclear).

Gel Electrophoresis—Gel electrophoresis was carried out according to the method (18) using 18% acrylamide gels with a thickness of 1.5 mm. A stacking gel of 4.6% acrylamide and 0.37% bisacrylamide was used. Samples were taken up in 0.2% SDS, 10% glycerol, 25 mM Tris, 2% 2-mercaptoethanol, and placed in a boiling water bath for 5 min. Electrophoresis was carried out at 40 mA until the sample was into the stacking gel and then increased to 60 mA for the duration of the electrophoresis. Acidurea gelatin gels were run according to Allfrey et al. (19), using a stacking gel of 7.5% acrylamide and 0.125% methylenebisacrylamide.

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modifed and allelic forms is provided by gel electrophoresis under acid conditions in the presence of urea and triton as described by Alflageme et al. (19). In the case of H3, there are two allelic forms of the histone represented as H3.1 and H3.2 in Fig. 5. The slower moving form (3.1) contains a cysteine at position 96, whereas the faster migrating band (3.2) contains a serine at that position (26). In all other positions, the sequences of 3.1 and 3.2 are identical. This conservation of sequence in H3 is maintained in most species with only 5 of 96 residues being variable (27). Each histone is resolved into a set of bands that represent different modification states of the histone such as acetylation or ADP-ribosylation. Nevertheless, it is clear from the gels shown in Fig. 5 that the only bands to shift mobility and migrate more slowly following phosphorylation of the nucleosome core with catalytic subunit are the H3 bands and that both allelic forms of H3 appear to be modified to an equal extent. The fact that the entire band has shifted mobility indicated that the phosphorylation of each histone 3 is complete.

In order to ascertain whether the specific phosphorylation of H3 was due to complex formation with DNA or whether that specificity would also be preserved in the histone octamer, phosphorylation of the nucleosome core was carried out in 2 mM NaCl. Under these conditions, the DNA and histones are not complexed and the core histones exist as a stable DNA-free octamer (28). As indicated in Fig. 2F, phosphorylation under these conditions reverted, in part, to the specificity seen

masked potential sites of phosphorylation on both H4 and H2B.

When the individual histones were characterized for the incorporation of radioactivity in long chromatin where histone 1 is still associated with the chromatin, the results (Fig. 4, upper panel) indicated that H1 was also phosphorylated. In this case, the amount of radioactivity incorporated into H1 was approximately half that incorporated into H3. This ratio presumably reflects the fact that H3 and H1 are present in molar ratios of 2:1 in H1-containing long chromatin (25) if in fact each molecule has only one site of phosphorylation.

A more detailed description of the histones and their various

FIG. 2. Polyacrylamide gel electrophoresis of phosphorylated histone mixture, long chromatin, nucleosome core, and H3-H4 tetramer. Phosphorylations were carried out in 0.1 ml of 50 mM Tris (pH 7.0) at a protein concentration of 1 mg/ml. Aliquots of 20 μl were used for electrophoresis. A, indicates Coomassie stain of the four core histones. The slowest migrating histone is histone 3, designated as 3. The other histones (2b, 2a, and 4) are indicated in increasing order of mobility. B, an autoradiogram indicating the phosphorylation of a mixture of histones (Sigma) following incubation for 5 and 60 min with catalytic subunit. The arrows in all cases (B-D) refer to the positions of each of the histones on the gel as identified by Coomassie staining. C, an autoradiogram showing the phosphorylation of tetramer containing H3 and H4. D, an autoradiogram showing the phosphorylation of long chromatin for 5 and 60 min. An autoradiogram showing the phosphorylation of nucleosome core under standard buffer conditions is shown in E for 2, 5, 10, 30, and 60 min whereas F indicates the phosphorylation of the same nucleosome core sample for 2 h in the presence of 2 mM NaCl.

FIG. 3. In vitro phosphorylation of the individual core histones by the catalytic subunit of CAMP-dependent protein kinase. A mixture of core histones was phosphorylated under conditions identical with those described in Fig. 1. Aliquots were removed at the designated time intervals and electrophoresed. After drying the gels, individual core histone bands were cut out and counted. Individual histones are represented as follows; histone 2B (○—○), histone 4 (□—□), and histone 3 (△—△).

FIG. 4. In vitro phosphorylation of nucleosome core and H1-containing long chromatin by the catalytic subunit of CAMP-dependent protein kinase. Long chromatin containing bound H1 was isolated as described under "Experimental Procedures." Long chromatin or nucleosome core (0.05 mg of protein) was incubated with 3 μg of catalytic subunit in a final volume of 0.25 ml containing 0.6 mM [γ-^32P]ATP and 2.0 mM MgCl2. Aliquots of 40 μl were removed at the designated times and subjected to SDS-polyacrylamide gel electrophoresis. Individual histone bands were cut out and counted from dried gels. Incorporation of ^32P into histones derived from long chromatin is indicated in the upper panel whereas incorporation into the histones of the nucleosome core is indicated in the lower panel.
in the free histone mixtures with radioactivity preferentially being incorporated into H2B. However, in contrast to the mixture of free histones, no incorporation of radioactivity into histone 4 was observed. This lack of histone 4 phosphorylation could also be due to partial interaction of DNA with the octamer since phosphorylation of the free histones in 2 M NaCl showed the same labeling pattern as in low ionic strength buffer. This alternative could best be resolved by isolating intact histone octamer free of DNA. Nevertheless, phosphorylation in the presence of 2 M NaCl clearly demonstrates that formation of an octamer alone is not sufficient to mask the accessibility of H2B to phosphorylation. At low ionic strength and at acidic pH, the core histones can be resolved into a tetramer containing H3 and H4 and an H2A:H2B dimer (14). When the isolated tetramer, H3:H4, was phosphorylated, equivalent amounts of radioactivity were incorporated into both H3 and H4 (Fig. 2C). In this case, the total amount of radioactivity incorporated into the tetramer was 3.7 mol of $^{32}$P per mol of tetramer.

As a preliminary step to identifying how many unique modification sites the kinase recognized, two-dimensional peptide maps were carried out on tryptic peptides prepared from histones labeled under a variety of conditions. As indicated in Fig. 6A, when the total mixture of histones was utilized, at least 5 unique radioactive peptides were visualized. In contrast, when the histones were isolated from long chromatin that had been phosphorylated under conditions where only H3 was labeled, peptide mapping (Fig. 6B) revealed only two closely aligned spots designated together as 3. The tetramer containing histones 3 and 4 was also phosphorylated and mapped. As seen in Fig. 6D, in addition to the doublet, 3, seen in Fig. 6B for long chromatin, another more acidic pair of peptides was obtained that together are designated as 4. Since SDS-polyacrylamide gels of the intact tetramer (Fig. 2C) indicated that both H3 and H4 were phosphorylated to roughly equivalent extents, this additional peptide could be assigned to H4. The other radioactive peptides were derived from H2B and are designated as 2 in Fig. 6A.

Following peptide mapping, the specific site or sites of modification in histone 3 were determined. Isolated H3 from calf thymus revealed the same labeling pattern as the H3 that was labeled in long chromatin based on peptide mapping (Fig. 6C). The mapping of purified phosphorylated H3 was carried out following phosphorylation of 6 mg of calf thymus H3 under the conditions described in Fig. 6. In addition, long chromatin from beef kidney was radiolabeled under the conditions described in Fig. 4. Based on the standard assay, 0.7 mol of $^{32}$P, was incorporated per mol of histone 3. Hydrolysis of phosphorylated H3, long chromatin, and also H3:H4 tetramer followed by paper electrophoresis at pH 2.1 indicated that in all instances only a serine was being modified. After extensive dialysis against water at 4°C, both samples were lyophilized and coupled to DITC-glass as described under “Experimental Procedures.” Sequencing was carried out on a solid phase Sequencer under conditions described previously (22). The results shown in Fig. 7 and Table I indicated that radioactivity was associated with serine 10 in both cases.

Since peptide mapping (Fig. 6) usually revealed that two major radioactive peptides were derived from H3-labeled histones following proteolysis with trypsin, the tryptic peptides were actually isolated in order to establish whether the two spots were due to bonds that were partially resistant to tryptic cleavage or whether a second site further down the poly-peptide chain was being modified in addition to serine 10. The two tryptic peptides can be more definitively resolved by
Experimental Procedures. The identity and yields of phenylthiohydantoin (PTH) amino acid at each step are shown. The release of radioactive peptides eluted together in a peak that immediately preceded the \[^{32}P\]ATP. Fractions containing radioactive H3 were pooled, lyophilized, and further resolved by high performance liquid chromatography. As indicated in Fig. 9, the composition of this peptide (Table II) corresponds to residues 36 to 48 with serine 38 being the site of modification. These results are consistent with the earlier work of Langham on H1 isolated from trout testis who demonstrated that serine 40 in that homologous histone is the site that is phosphorylated by the catalytic subunit of cAMP-dependent protein kinase (29).

Some of the nonhistone chromosomal proteins were also found to be substrates for this kinase. In particular, the susceptibility of the purified HMG proteins to modification was also observed in some instances although it was present in insufficient amounts to characterize. Since it was not consistently observed, it was assumed to be an additional product of incomplete tryptic cleavage.

In addition to labeling the core histones, several other proteins were found to serve as in vitro substrates for cAMP-dependent protein kinase. As indicated in Fig. 4, if H1 was present in addition to the core histones, it was also a good substrate for the catalytic subunit. Labeling of H1 followed by paper electrophoresis of the tryptic peptides indicated a single major radioactive peptide. This peptide was isolated by gel filtration as described previously for H3 and purified to homogeneity by high performance liquid chromatography (Fig. 9). The composition of this peptide (Table II) corresponds to residues 36 to 48 with serine 38 being the site of modification. These results are consistent with the earlier work of Langham on H1 isolated from trout testis who demonstrated that serine 40 in that homologous histone is the site that is phosphorylated by the catalytic subunit of cAMP-dependent protein kinase (29).

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Phosphorylation by CAMP-dependent Protein Kinase

FIG. 9. Isolation of the phosphorylated tryptic peptides from H3 and H1 by high performance liquid chromatography. Following gel filtration, the phosphorylated peptides obtained from tryptic digestion of H3 and H1 were pooled, lyophilized, and separated by high performance liquid chromatography. Fractions were monitored for absorbance at 219 nm, and aliquots were counted for radioactivity. The fractions containing \(^{32}P\)-radioactivity are indicated.

TABLE II
Phosphorylated tryptic peptides isolated from H3 and H1 by high performance liquid chromatography

The peptides were isolated by high performance liquid chromatography as shown in Fig. 9. Compositions are given in molar ratios. Theoretical values of the corresponding H3 and H1 peptides are indicated in parentheses.

<table>
<thead>
<tr>
<th>Amino acid</th>
<th>Peptide</th>
</tr>
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<tbody>
<tr>
<td>Aspartate</td>
<td>3A 1.0(1)</td>
</tr>
<tr>
<td>Threonine</td>
<td>3B 1.0(1)</td>
</tr>
<tr>
<td>Serine</td>
<td>1A 0.7</td>
</tr>
<tr>
<td>Glutamate</td>
<td>3A 0.9(1)</td>
</tr>
<tr>
<td>Proline</td>
<td>3B 2.0(2)</td>
</tr>
<tr>
<td>Glycine</td>
<td>3C 2.1(2)</td>
</tr>
<tr>
<td>Alanine</td>
<td>3D 1.1(1)</td>
</tr>
<tr>
<td>Valine</td>
<td>3E 1.0(1)</td>
</tr>
<tr>
<td>Leucine</td>
<td>3F 0.9(1)</td>
</tr>
<tr>
<td>Lysine</td>
<td>3G 1.0(1)</td>
</tr>
</tbody>
</table>

Fig. 10. In vitro phosphorylation of HMG 14 and 17 by the catalytic subunit of cAMP-dependent protein kinase. Samples contained 5 nmol of HMG 14 and 10 nmol of HMG 17 in a total volume of 0.1 ml. Phosphorylation conditions were as described under "Experimental Procedures." Aliquots of 0.015 ml were removed at the designated time intervals and subjected to SDS-polyacrylamide gel electrophoresis. After staining and destaining, the gels were dried and counted.

of HMG 17. In the experiment indicated in Fig. 10, 0.85 nmol of \(P\), was incorporated per mol of HMG 14. Peptide maps of HMG 14 revealed several labeled tryptic peptides; however, it was not established whether this was due to modification at multiple sites or whether a single residue was modified, but digestion with trypsin was simply incomplete as was seen with H3.

Knowing that both H1 and HMG 14 and 17 bind to chromatin, the effect of those proteins on H3 phosphorylation was investigated. A mixture of HMG 14 and 17 had no effect on the phosphorylation of H3. In contrast, addition of H1 to long chromatin that had been depleted of H1 by gel filtration in the presence of 0.6 M NaCl showed significant inhibition of H3 phosphorylation (Fig. 11) when H1 was present in slight excess over the core histones. Addition of a greater than 3-fold excess of purified H1 over core histones actually resulted in exposure of the H2B sites to phosphorylation indicating significant disruption of the core structure. Whether such an effect has physiological significance remains to be established as does the exact stoichiometry of H1 needed to inhibit H3 phosphorylation.

DISCUSSION

The structure of chromatin as consisting of nucleosome cores was first described by Kornberg (25) and has been recently reviewed by McGhee and Felsenfeld (30). In long chromatin, each histone octamer containing equivalent amounts of the four core histones (H3, H4, H2B, and H2A) is surrounded by two turns of a supercoiled DNA double helix. In a single isolated nucleosome core, the histone octamer is surrounded by 1.75 turns of the supercoiled DNA which contains 140-148 base pairs. Both forms, long chromatin and nucleosome core, represent the native state in which histones exist in the nucleus of the cell. When phosphorylation of free histones by the catalytic subunit of cAMP-dependent protein kinase is compared to phosphorylation of chromatin or nucleosome core, the differences are striking. Multiple sites are accessible and readily phosphorylated in the free histone mixture. We have demonstrated that histone 3 is modified at serine 10. Histone 4 is also phosphorylated at a serine residue and, although the site of modification was not sequenced, the electrophoretic mobility at pH 6.5 of the phosphorylated tryptic peptides derived from H4 (Fig. 6D) and their rapid
mobility in the chromatography dimension strongly suggest that the modification occurred at serine 47. Two sites are phosphorylated in histone 2B, and these were identified previously as serine 32 and 36 (5). Of these four sites that are readily phosphorylated in mixtures of free histones, only one, serine 10, remains accessible to the kinase when the histones are part of a DNA complex.

The phosphorylatable serine in histone 3 at position 10 is located in a region of the molecule that is readily accessible to a variety of other modifications including acetylation at lysines 9, 14, 18, and 23, and methylation at lysines 9 and 27 (31). Presumably some of these modifications such as acetylation of lysine 9 would have a marked effect on the phosphorylation of serine 10 since the catalytic subunit characteristically recognizes two basic residues preceding the site of phosphorylation. Histone 3 is also readily susceptible to limited proteolysis by endogenous proteases as well as in vitro proteolysis by a variety of proteases. The site of cleavage by endogenous proteases is at lysine 23 (32). These multiple modifications further emphasize that this region of the molecule is highly accessible in chromatin. It is not known what role these modifications play in the overall functioning of chromatin. It is known, for instance, that active genes contain histones 3 and 4 in highly acetylated forms (33). In addition, phosphorylation of H3 is associated with mitotic chromosomes, and this is the only time in the cell cycle when H3 is phosphorylated (34). None of the other core histones are phosphorylated during mitosis. However, in the absence of any biological assays, it is difficult to assess the physiological significance of any specific individual modification. It is possible that phosphorylation could be a protective mechanism against proteolysis or acetylation. On the other hand, phosphorylation could trigger chromosome condensation prior to mitosis as suggested by Bradbury et al. (35). Clearly, introducing a negatively charged phosphate group into a molecule that is complexed with highly negatively charged DNA could result in a significant perturbation.

Each core histone can be considered as a globular molecule with a somewhat extended highly basic NH₂-terminal region (36). The H3 modifications described above all occur in the nonglobular region of the molecule. In addition to serine 10 on H3, the other sites of modification in the free histone mixture are associated with H2B and H4. The H2B sites are also in the basic NH₂-terminal region of the molecule, whereas the site of phosphorylation in H4 is more in the middle of the molecule just beyond the basic region. The H2B sites of phosphorylation are exposed in the histone mixture even when the histones exist as a stable octamer in high salt but are masked when DNA is added to the complex. The H4 site of phosphorylation clearly is exposed in the free histone mixture but may be masked in the stable octamer. The absolute specificity of the CAMP-dependent protein kinase for serine 10 of histone 3 in chromatin appears to be a sensitive criterion for assessing the integrity of the chromatin or of the nucleosome core, since disruption of the chromatin structure by procedures such as freezing and thawing, high salt, or simply extended storage result in exposure of the additional peripheral sites of phosphorylation. In the intact nucleosome core only negligible phosphorylation of H2B, H2A, and H4 was observed under conditions where H3 phosphorylation was carried out to completion. This was in contrast to earlier work which showed a clear preference for H3 phosphorylation over phosphorylation of the other core histones but not an absolute specificity (37).

The phosphorylation of H1 is more complex for although the catalytic subunit of CAMP-dependent protein kinase phosphorylates only one site, there are known to be multiple sites of phosphorylation by other endogenous kinases in the nucleus (38). Furthermore, the site that is phosphorylated in H1 is not a conserved serine in contrast to serine 10 in histone 3. For those reasons, it is extremely difficult to assess the possible significance of this single phosphorylation site.

The phosphorylation of HMG 14 is also of potential interest since this protein, together with HMG 17, is thought to be associated with actively transcribing DNA (39).

Although the physiological significance of histone phosphorylations is still unclear, it has been established that phosphorylation of histone 3 and of certain H1 sites are associated uniquely with mitotic chromosomes (34). Consequently, having established specific sites of in vitro phosphor-
Chromatin Phosphorylation by cAMP-dependent Protein Kinase

REFERENCES


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by the catalytic subunit of cAMP-dependent protein kinase in one of the core histones, H3, and in H1, and having shown that HMG 14 is also phosphorylated by the catalytic subunit, it was of interest to compare these phosphorylations to physiological chromatin phosphorylations that are associated uniquely with chromosomes synchronized in mitosis. The following paper will compare these in vivo phosphorylations with the in vitro phosphorylations described here.
The in vitro phosphorylation of chromatin by the catalytic subunit of cAMP-dependent protein kinase.

S S Taylor


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