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Modulation of Nuclear Matrix Protein Phosphorylation by Histones: Possible Involvement of NM-Associated Protein Kinase CK2 Activity

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Abstract Nuclear matrix (NM), a proteinaceous network of filaments, dictates nuclear morphology and the structure/function of DNA. Phosphorylation of NM proteins is a potential signal for regulating matrix functions. Histones also are intimately involved in DNA structure and transcription. Here, we report that various histones enhanced ^{32}P incorporation into certain NM proteins. Modulation of NM protein phosphorylation by histones is mediated through regulation of protein kinase CK2, a messenger-independent serine/threonine kinase, which is significantly associated with the NM. The stimulatory effect of histones was mitigated by prior incubation of histones with DNA in the reaction. Phosphorylation of NM proteins was extensively reduced when an excess of the CK2-specific peptide substrate was included in the phosphorylation reaction as a competitor. Also, enhancement in the NM-associated CK2 activity by histones was blocked by inhibitors of CK2. Histone H1 effect appeared to be mediated mainly by charge effect since a stretch of polylysine induced a similar effect. Various histones also differentially affected the autophosphorylation of NM-associated CK2 subunits. This may contribute to the observed effects of histones on the NM, resulting in an enhancement and differential pattern of NM protein phosphorylation. Such a regional modification of NM protein phosphorylation might influence the nuclear functions that require histone displacement, namely, replication and transcription. J. Cell. Biochem. 72:242–250, 1999. Published 1999 Wiley-Liss, Inc.†

Key words: protein kinase CK2; nuclear matrix; histones; DNA; protein phosphorylation

The genomic DNA of higher eukaryotic cells appears to be organized into loops of chromatin, restrained at their bases by interaction with the nuclear matrix (NM) [Getzenberg et al., 1991]. Discrete segments of DNA are involved in matrix binding; these segments have been designated matrix attachment regions (MARs). There is increasing evidence that this nonrandom organization of DNA is of functional significance. For example, DNA origins of replication, as well as the regulatory elements of many actively transcribed genes, are localized with, or juxtaposed to, these MARs [Gasser and Laemmli, 1989; Hozak et al., 1993; Forrester et al., 1994]. Furthermore, by linking MAR sequences to appropriate constructs, expression of transfected genes is enhanced [Stief et al., 1989; Blasquez et al., 1989]. This transcriptional advantage conferred on sequences of DNA that attach to the matrix might be attributed to several factors. First, torsional stress, which maintains DNA supercoiling, enhances transcription in vitro [Villeponteau et al., 1984]. Second, DNA attached or in close proximity to

Abbreviations used: NM, nuclear matrix; CK2 or protein kinase CK2, casein kinase 2; MARs, matrix attachment regions; Poly(GT), poly(Glu,Tyr) (4:1); DRB, 5,6-dichlororibofuranosylbenzimidazole; IF, intermediate filaments; Mes, 2-[N-morpholino]ethanesulfonic acid; PMSF, phenylmethylsulfonyl fluoride; BSA, bovine serum albumin; SDS, sodium dodecyl sulfate; PAGE, polyacrylamide gel electrophoresis.

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the matrix is exposed to many matrix-associated proteins that are essential for DNA replication and transcription. These include DNA polymerases, primase, topoisomerase I and II, several transcription factors, cell signaling molecules, and oncoproteins [Wood and Collins, 1986; Ackerman et al., 1988; Tawfic and Ahmed, 1994a; van Wijnen et al., 1993; Cook, 1991; Mancini et al., 1994]. Thus, the local concentration of all these proteins and the regulatory sequences of different genes at the NM may explain how, with low representation of these factors and sequences in the nucleus, a threshold to initiate replication and transcription can be obtained in vivo.

Histones are the primary proteins whose properties mediate the folding of DNA into polynucleosomes. Transcriptional regulation of a broad range of genes is modulated by both the stoichiometry and the individual domains of histone proteins [Wolffe, 1994]. It is widely accepted that replication and transcription require disruption of the nucleosome; several models have been proposed for this process [Bresnick et al., 1992; Felsenfeld, 1992; Brooks and Jackson, 1994; Lewin, 1994]. However, in almost all models, the release of histone H1 is necessary for further nucleosome displacement. This implies a transient exposure of the positively charged domains of free histone H1 as well as interaction with surrounding proteins. Studying the outcome of the interaction between histones and NM proteins is therefore central to delineation of the structure–function relationship of nuclear architecture.

We have previously reported that NM serves as an anchor for protein kinase casein kinase 2 (CK2) and that several of the NM-associated proteins are phosphorylated by CK2 [Tawfic and Ahmed, 1994a,b]. Further, it appears that CK2 is dynamically regulated in the nucleosomes in relation to transcriptional activity [Guo et al., 1998]. Considering the involvement of CK2 in the NM and chromatin functions, we hypothesized that direct interaction between histones and NM proteins during gene activities might modify this intrinsic kinase activity, and consequently the state of phosphorylation of local proteins. In this paradigm, the matrix-DNA attachment regions, where transcription and replication are taking place, would be distinct from nonattachment sites with respect to their phosphorylation status. Such a distinction might be involved in the regional modification of protein–protein interactions or the catalytic activity of proteins essential for the functional activities attributed to those sites.

To test this hypothesis, we ascertained whether histones were able to modulate the phosphorylation of NM proteins. We have found that, to differing degrees, purified histones were able to modulate the phosphorylation of NM proteins both quantitatively and qualitatively. Histone H1 evoked the highest enhancement of ^{32}P incorporation into NM proteins, although all other histones were also active. This effect appears to be directly mediated by the lysine residues of histone H1 and can be reversed by prior addition of DNA. The phosphorylation pattern of isolated NM proteins phosphorylated in vitro is different from that observed when they are phosphorylated in intact nuclei or cells; however, the addition of histones to isolated NM induced a phosphorylation pattern very similar to that observed in the latter case. The modulation of NM protein phosphorylation by histones could be inhibited by inhibitors specific for CK2, indicating a role for the NM-CK2 in this process. In addition, various histones differentially modulated phosphorylation of the α- and β-subunits of NM-associated CK2.

The phosphorylation status of CK2 subunits has been suggested to play a role in determining its substrate specificity [Litchfield et al., 1992]. Thus, our results suggest that, in any given state of cell activation, the differential phosphorylation of CK2 subunits evoked by the presence of different histones might be a significant factor in determining the substrate specificity of intrinsic CK2, thereby dictating the subset of NM proteins to be phosphorylated.

MATERIALS AND METHODS

Animals

Male Sprague-Dawley rats weighing 295–325 g (Harlan Sprague-Dawley, Indianapolis, IN) were used as the source of liver tissue. The animals were maintained under standard conditions as described previously [Tawfic and Ahmed, 1994a].

Chemicals

Purified histones H1, H2A, and H2B and arginine-rich histones H3/H4 (i.e., H3 + H4) were obtained from Worthington Biochemical Corp. (Freehold, NJ). (The molecular weights of these various histones were 21.5, 14, 13.8, 15, and 11.3 kDa, respectively.) The specific deca-

peptide substrate (Arg-Arg-Arg-Glu-Glu-Glu-Thr-Glu-Glu-Glu) for assaying CK2 activity was purchased from Cambridge Research Biochemical (Wilmington, DE). Poly(GT), Polylysine (M_r 35,000), and 5,6-dichloro-ribofuranosylbenzemidazole (DRB) were purchased from Sigma Chemical Co. (St. Louis, MO). Antibody against CK2 was as described before [Tawfic and Ahmed, 1994a]. DNase I and RNase A were purchased from Boehringer Mannheim (Indianapolis, IN). All common reagents and chemicals were of the highest purity available.

Preparative Methods

Preparation of Nuclear Matrix. Nuclei were prepared from pooled liver tissue as described previously [Tawfic and Ahmed, 1994a]. Purified nuclei were depleted of soluble and DNA- and RNA-associated proteins including histones by sequential extractions in buffers containing detergents, high salt, and nucleases as described previously [Tawfic and Ahmed, 1994b]. The remaining NM-intermediate filament (IF) fraction was then suspended in a disassembly buffer (8 M urea, 20 mM Mes, 1 mM EGTA, 0.1 mM $MgCl_2$, 1% 2-mercaptoethanol, and 1 mM phenylmethylsulfonyl fluoride (PMSF), final pH 6.6) and dialyzed overnight at 4°C against a 2,000-fold excess of the assembly buffer (0.15 M KCl, 25 M imidazole HCl, 5 mM $MgCl_2$, 2 mM dithiothreitol, 0.125 mM EGTA, and 0.2 mM PMSF, final pH 7.1). The reassembled IF were pelleted by centrifugation at 150,000*g* for 90 min at 4°C, and the IF-depleted NM fraction was recovered from the supernatant fluid. The protein content of the NM fraction was determined by using the Bio-Rad protein assay. All the general characteristics of the isolated NM fraction were as reported previously [Tawfic and Ahmed, 1994b].

Assays

^{32}P incorporation into NM proteins. The NM fraction was used as the source of both the protein kinase activity and substrates. The NM proteins were incubated in a phosphorylation reaction mixture optimum for studying messenger-independent kinase systems, which contained 30 mM Tris-HCl, pH 7.45, 5 mM $MgCl_2$, 1 mM dithiothreitol, 20 mM NaCl, 50 mM β-glycerophosphate, 4 µM Microcystin-LR, 0.5 mM PMSF, 1.0 µg/ml leupeptin, and 10 µM [γ-^{32}P]ATP (specific activity 30×10^6 dpm/nmol) with or without different histones at 37°C for the periods of time indicated in the figure legends. Histones were added in a ratio of 3:1 with respect to NM proteins, the relative concentration at which histones induced the maximal effects. In the control tubes, histones were replaced with equivalent amounts of bovine serum albumin (BSA). Whenever indicated, calf-thymus DNA at different concentrations was mixed with purified histone H1 in a buffer containing 10 mM Tris-HCl, pH 8, 35 mM NaCl, 0.1 mM dithiothreitol, and 0.03% Nonidet P-40 (NP-40) and incubated at 8°C for 30 min before starting the kinase reaction [Levine et al., 1993]. The ratio between added DNA and histone H1 was adjusted on the basis of the observation that a ratio of 1:0.6 is adequate to abolish transcription in vitro [Levine et al., 1993]. The kinase reaction was stopped by adding cold 15% trichloroacetic acid, and the ^{32}P incorporated into NM proteins was estimated using the precipitation method described previously [Tawfic and Ahmed, 1994b].

To measure the effect of different histones on the pattern of phosphorylation of intrinsic NM proteins, equal amounts of the proteins were incubated for 10 min at 37°C in the above-described phosphorylation reaction in the presence or absence of a histone. Controls contained an equivalent amount of BSA instead of histones. Polylysine was added in an amount equivalent to the number of lysine residues in histone H1, which was based on the molecular weight of histone H1, 21.5 kDa; polylysine, 35 kDa; and lysine, 146.2 Da, and on the fact that histone H1 has about 66 lysine residues among its 223 amino acids [Strickland et al., 1976]. After incubation for 15 min at 37°C, the proteins were precipitated by adding cold 15% trichloroacetic acid. The precipitate was washed three times with the same solution and finally with absolute ethanol. The pellet was dried, dissolved by boiling in sample buffer containing 10 mM sodium phosphate, pH 7, 8 M urea, 5% 2-mercaptoethanol, and 5% sodium dodecyl sulfate (SDS) and subjected to polyacrylamide gel electrophoresis (PAGE) on a 7.5–20% gradient polyacrylamide GelBond slab gel containing 0.1% SDS. The gel was stained with silver, dried, and exposed overnight to Kodak X-Omat AR film at −70°C. In another approach, the proteins on the gel were transferred to nitrocellulose paper and probed with the polyclonal antibody against CK2 as described previously [Tawfic and Ahmed, 1994b].

Heparin, poly(Glu, Tyr) [poly(GT)], and 5,6-dichloro-ribofuranosylbenzimidazole (DRB) were

employed as inhibitors of CK2 at the concentrations indicated in the figure legend to the above mentioned kinase reaction with or without histones, and the ^{32}P incorporated into NM proteins was estimated using the precipitation method described previously [Tawfic and Ahmed, 1994b]. Experiments were also included in which CK2-specific peptide substrate was added as a competitor for phosphorylation of NM proteins catalyzed by intrinsic CK2.

Phosphorylation of CK2-specific peptide substrate catalyzed by CK2 intrinsic to NM. Equal amounts of NM protein were incubated in the above-described phosphorylation reaction medium in the presence or absence of the CK2-specific peptide substrate. The amount of ^{32}P incorporated into the substrate in the presence or absence of different histones was determined by the paper binding method described previously [Tawfic and Ahmed, 1994a,b].

Immunoprecipitation of NM-associated CK2. The chicken polyclonal antibody immunopurified against protein kinase CK2 was bound to activated Sepharose-4B beads, using cyanogen bromide as described previously [Tawfic et al., 1993]. The NM proteins, in the presence or absence of different histones or polylysine, were incubated for 15 min at 37°C as described above. The reaction was stopped by adding an 8-fold excess of cold buffer containing 0.35 M sucrose, 3 mM sodium phosphate, 1 mM sodium pyrophosphate, and 50 mM β-glycerophosphate as phosphatase inhibitors. The reaction medium in each case was incubated with Sepharose-bound CK2 antibody for 6 h at 4°C in a buffer containing 10 mM Tris-HCl, pH 7.4, 150 mM NaCl, and 50 mM β-glycerophosphate. The bound material was washed four times with 10 mM Tris-HCl, pH 7.4, 100 mM NaCl, and 50 mM β-glycerophosphate then eluted with a buffer containing 10 mM Tris-HCl, pH 7.4, 4 M $MgCl_2$, and 50 mM β-glycerophosphate and washed four times with the same medium. BSA (500 μg) was added to the eluate as a carrier, and the mixture was precipitated with 15% trichloroacetic acid, washed with absolute ethanol, and dried. The dried sample was dissolved in sample buffer and subjected to SDS-PAGE as described above. The gel was silver stained to quantitate the protein kinase CK2 eluted from the column in each case. The dried gel was exposed on Kodak X-Omat AR film overnight, and the relative densitometric values of protein kinase CK2 apparent in silver stain as well as the autoradiogram were quantitated using a Bio-Rad densitometer.

RESULTS

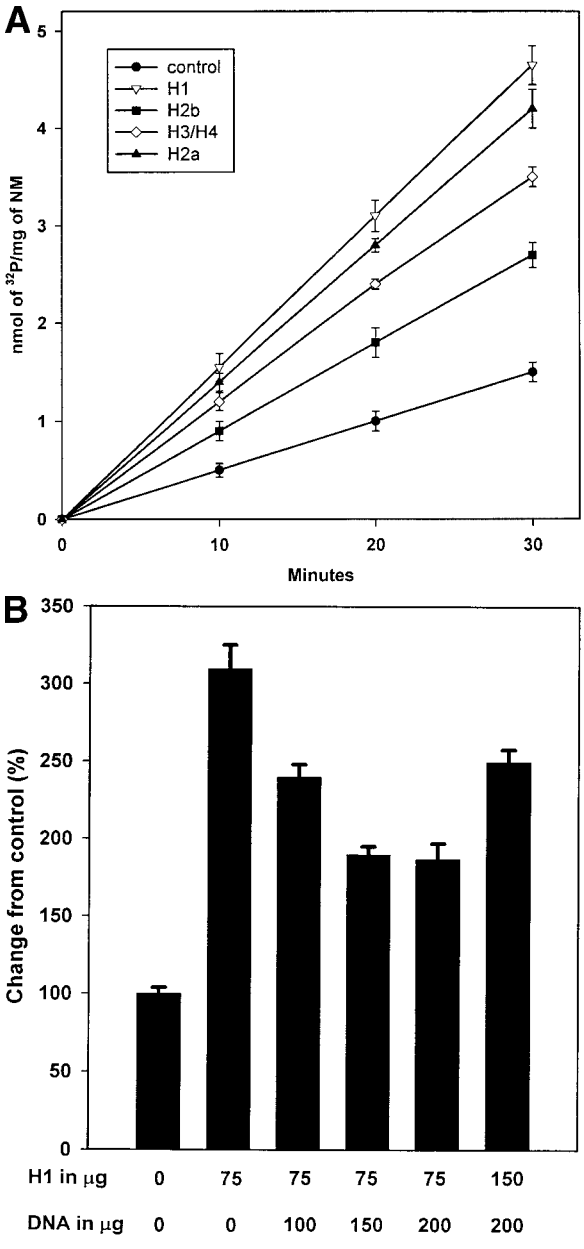
Effect of Various Histones on Phosphorylation of NM Proteins Mediated by the Intrinsic Kinase

Considering that histones may play a role in the activity of protein kinase intrinsic to the isolated NM during gene activity, we investigated the effects of purified histones on this system. The addition of various purified histones to the NM fraction led to a differential increase in the rate of incorporation of ^{32}P into the NM proteins catalyzed by the kinase activity intrinsic to that fraction (Fig. 1A). Histone H1 showed a higher capacity to stimulate the phosphorylation of NM proteins (about 3-fold stimulation) followed, in descending order of activity, by histones H2A, H3/4, and H2B (Fig. 1A). This enhancement of ^{32}P incorporation was not ascribable to a stabilization of the endogenous kinase activity by these proteins, as the addition of an equivalent amount of albumin to control tubes had no effect. Quantitatively, this enhancement of ^{32}P incorporation into NM proteins evoked by histone H1 was reduced by preincubation of histone H1 with DNA. Presumably, this effect was a result of the binding of histone H1 to DNA, because it could be reversed by further addition of H1 (Fig. 1B).

Effect of Different Histones on the Pattern of NM Protein Phosphorylation

Under the reaction conditions used, there was no ^{32}P incorporation into the different histones added to the reaction (Fig. 2A), which suggests that they served as a stimulator of the phosphorylation of endogenous proteins rather than as substrates for the NM-associated kinase activity. The addition of histones enhanced the incorporation of ^{32}P into certain NM proteins, and the extent of this enhancement varied with the type of histone protein added. That is, there were small but distinguishable differences between the patterns of NM phosphorylation mediated by different histones. All histones enhanced the incorporation of ^{32}P into proteins with apparent molecular weight of 180, 150, 28, and 10 kDa (Fig. 2A, lanes b, c, d, e, cf. lane a). The 28-kDa band corresponded to the β-subunit of CK2. In addition, histones H1 and H2A selectively enhanced ^{32}P incorporation into the protein with apparent molecular weight of 110 kDa (Fig. 2A, lanes b, c, cf. lane a), while

histones H2B and H3/H4 lacked this effect. Phosphorylation of NM proteins in the presence of histones H2B and H3/H4 showed a decline in ^{32}P incorporation in several proteins, such as the 63 and 52 kDa proteins (Fig. 2A, lanes d, e, cf. lane a). The pattern of NM protein phosphorylation in situ (i.e., in intact nuclei or intact cells) as described previously [Tawfic and Ahmed, 1994a] and in Fig. 2A (lane h) was different from that obtained when isolated NM proteins were subject to phosphorylation (Fig. 2A, lane h cf. lane a). Interestingly, the addition of histone H1 or H2A to the isolated NM fraction induced a pattern of phosphorylation similar to that observed in intact cells (Fig. 2A, lanes b and c cf. lane h).



Effect of Polylysine on NM Protein Phosphorylation

To ascertain whether modulation of NM protein phosphorylation by histone H1 was attributable to a charge effect mediated by its lysine-rich domains, we compared the effect of H1 with a stretch of polylysine containing approximately the same number of lysine residues present in H1. Addition of polylysine modulated the pattern and rate of ^{32}P incorporation into NM proteins in a comparable manner to that induced by histone H1 (Fig. 2A, lane f cf. lane b). However, unlike H1, polylysine enhanced ^{32}P incorporation into the protein with apparent molecular weight of 17 kDa.

To examine further the role of the charged domains of histone H1 in determining the pattern of NM protein phosphorylation, histone H1 was preincubated with DNA before initiation of the phosphorylation reaction. Qualitatively, preincubation led to a decline in ^{32}P incorporation into NM proteins and restored the pattern of NM protein phosphorylation seen without histones (Fig. 2A, lane g cf. lane a).

Involvement of Protein Kinase CK2 in H1-Mediated Enhancement of ^{32}P Incorporation into NM Proteins

We have previously demonstrated that a significant amount of nuclear CK2 is localized in the NM and that phosphorylation of several NM proteins is abolished by specific inhibitors

Fig. 1. **A:** Effect of various histones on the rate of ^{32}P incorporation into NM proteins catalyzed by the endogenous kinase activity. NM protein equivalent to 5 μg was incubated in the protein kinase reaction buffer in the presence of 15 μg of various histones indicated. The control contained 15 μg of bovine serum albumin instead of histone. After the indicated times, the proteins were precipitated by addition of cold 15% trichloroacetic acid, and the ^{32}P incorporated into proteins was determined by the precipitation method as described under Materials and Methods. The data are presented as mean ± SEM, and are based on triplicate experiments. **B:** Effect of DNA on enhancement of ^{32}P incorporation into NM proteins by histone H1. Calf-thymus DNA and histone H1 were preincubated for 30 min at 8°C in the Tris buffer (described under Materials and Methods) at the concentrations indicated. The endogenous kinase reaction was started by adding the kinase reaction buffer as described under Materials and Methods. The reaction was stopped after 5 min by the addition of cold 15% trichloroacetic acid, and the incorporated ^{32}P in NM proteins was estimated using the precipitation method described under Materials and Methods. Experiments were carried out in triplicate, and the data are presented as mean ± SEM.

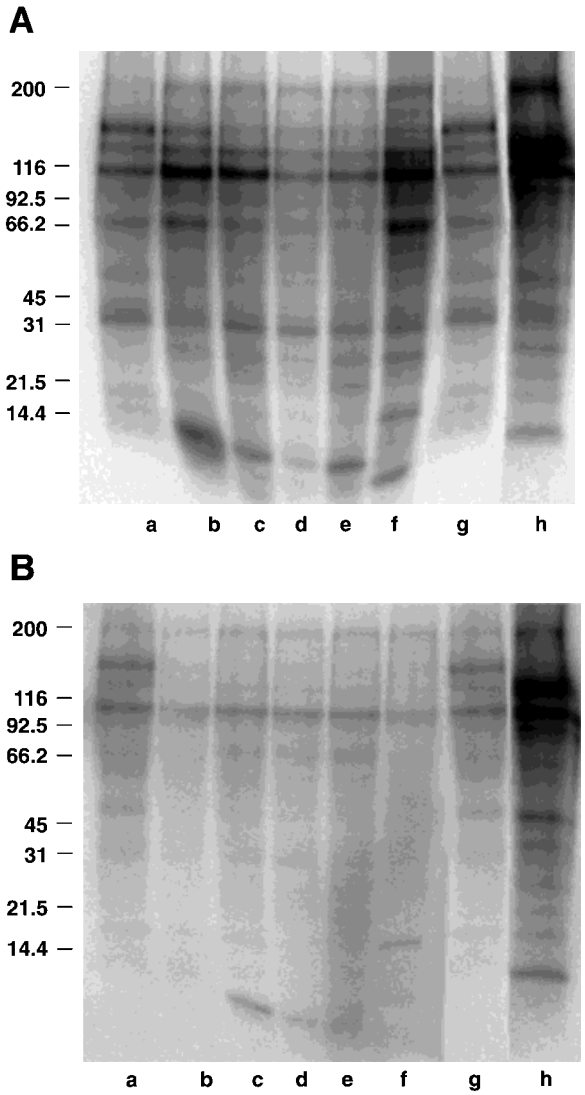


Fig. 2. Effect of various histones on the pattern of NM protein phosphorylation catalyzed by the NM-associated kinase activity. **A:** Autoradiogram of ^{32}P-labeled proteins in the NM fraction. **Lanes a, b, c, d, e,** isolated NM proteins (15 µg) phosphorylated in the presence of 45 µg each of BSA, histones H1, H2A, H2B, and H3/H4, respectively; **lane f,** NM proteins (10 µg) phosphorylated in the presence of 5.1 µM (18 µg) of polylysine; this amount of polylysine is added to provide approximately the same number of lysine residues in 45 µg (18.7 µM) of histone H1; **lane g,** NM proteins phosphorylated after addition of histone H1 (45 µg) preincubated with 135 µg of DNA as described under Materials and Methods; **lane h,** NM proteins phosphorylated in intact nuclei. **B:** Same as **A,** except that 1 mM CK2-specific peptide substrate was also included in the reaction as a competitor for phosphorylation of NM proteins.

of protein kinase CK2 [Tawfic and Ahmed, 1994a,b]. We therefore studied whether the enhancement of phosphorylation of NM proteins by histones involved modulation of intrinsic CK2 activity.

The data in Figure 2B show that the specific peptide substrate for CK2 could decrease the histone-mediated ^{32}P incorporation into NM proteins. In addition, protein kinase CK2 inhibitors were able to abolish completely the increase in ^{32}P incorporation evoked by histone H1 (Fig. 3A). On the other hand, the addition of different histones to the NM enhanced the rate of ^{32}P incorporation into the specific peptide substrate for CK2. Again, histone H1 was the most effective in enhancing ^{32}P incorporation into the CK2-specific peptide followed by histones H3/H4, H2A, and H2B (Fig. 3B).

Effect of Different Histones and Polylysine on the Autophosphorylation of NM-Associated CK2

If protein kinase CK2 acts upstream from the observed modulations in NM protein phosphorylation mediated by histones, changes in the phosphorylation of protein kinase CK2 subunits might be causally related to this modulation. To explore this possibility, we examined the effects of various histones on ^{32}P incorporation into subunits of NM-associated CK2. Using the Sepharose-bound specific antibody to immunoprecipitate the NM-associated protein kinase CK2 after labeling with ^{32}P in the absence or presence of histones, we found that histones and polylysine modulated the intensity of ^{32}P incorporation into both of the subunits of CK2 (Fig. 4). Each histone induced a reproducible specific pattern of phosphorylation of both subunits of the kinase (Fig. 4). Histones H1 and H2A induced essentially the same pattern of phosphorylation of the CK2 subunits (Fig. 4, lanes b and d), while histones H2B and H3/H4 induced a different pattern of phosphorylation of both subunits (Fig. 4, lanes e and f). Polylysine abolished the phosphorylation of CK2-β-subunit (Fig. 4, lane c).

DISCUSSION

This work provides evidence for modulation of both the level and the pattern of phosphorylation of NM proteins by histones. This effect appears to be mediated partly by the positively charged domains of histones, as it was diminished by preincubation of histones with DNA. However, the presence of some qualitative differences in phosphorylation of NM proteins in the presence of different histone (especially those rich in lysine) may suggest a role as well for direct histone–kinase and/or histone–substrate interactions in determining the quantitative as well as the qualitative differences in the phosphorylation of NM proteins. However, our data support the notion that modulation of NM

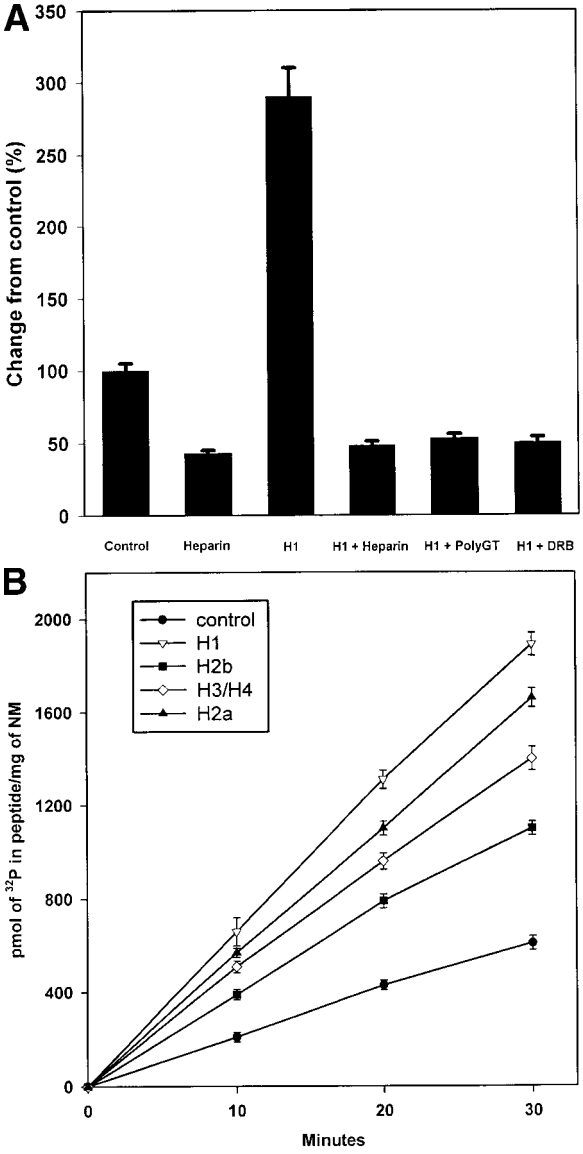


Fig. 3. **A:** Effect of protein kinase CK2 inhibitors on the histone H1 enhancement of ^{32}P incorporation into NM proteins. Isolated NM fraction (15 µg) was incubated in the protein kinase reaction medium in the presence of heparin 1 µg/ml, 45 µg of histone H1, or Poly(GT) 20 µg/ml, or 30 µM of DRB alone or in combinations as indicated. The kinase reaction was stopped by the addition of cold 15% trichloroacetic acid. The precipitated protein was washed, and the ^{32}P incorporated into NM proteins was estimated using the acid precipitation procedure as described under Materials and Methods. **B:** Effect of different histones on the incorporation of ^{32}P into protein kinase CK2-specific peptide substrate using the isolated NM fraction as the source of the kinase. Isolated NM fraction was incubated alone or in the presence of various histones with the specific peptide substrate. The amount of ^{32}P incorporated into the peptide substrate was determined by the paper binding method described under Materials and Methods.

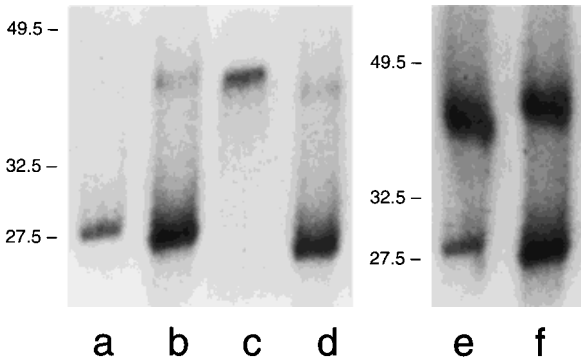


Fig. 4. Phosphorylation status of NM-associated protein kinase CK2 subunits in the presence of various histones and polylysine. NM fraction (containing the protein kinase CK2) was labeled with ^{32}P by incubation in the protein kinase reaction medium for 15 min in the absence or presence of different histones or polylysine, as described under Materials and Methods. Sepharose-bound anti-CK2 antibody was used to immunoprecipitate NM-associated CK2. **Lane a,** Immunoprecipitated NM-associated CK2 subunits α and β; **lanes b, c, d, e, f,** in the presence of H1, polylysine, H2A, H2B, and H3/H4, respectively. The amount of protein kinase CK2 eluted from the column in each case was the same as assessed by silver staining (data not shown).

protein phosphorylation by histones is due, at least in part, to the direct interaction between histones and CK2, which is present in a significant amount in the NM, where it is involved in the phosphorylation of many intrinsic proteins [Tawfic and Ahmed, 1994a,b]. First, the rate of enhancement of ^{32}P incorporation into CK2-specific peptide substrate by different histones was comparable to that induced by histones in NM proteins (Figs. 1A, 3B). Second, the effect of histones on NM protein phosphorylation was abolished by the specific peptide substrate of protein kinase CK2 (Fig. 2B). Other commonly employed inhibitors of CK2 such as heparin, poly(GT), and DRB also induced similar effects (Fig. 3A). Finally, purified histones markedly influenced the phosphorylation pattern of the subunits of CK2. It is noteworthy that autophosphorylation of purified protein kinase CK2 has been observed to be modulated by purified histones in vitro [Palen and Traugh, 1991].

The pattern of NM phosphorylation in the isolated NM fraction differs from that obtained when intact cells or nuclei are incubated with radiolabeled ^{32}P [Tawfic and Ahmed, 1994b]. This may imply that some non-NM proteins contribute to the pattern of phosphorylation obtained in vivo (i.e., in intact cells or nuclei). Interestingly, addition of H1 or H2A to isolated NM induced a pattern similar to that obtained in intact cells or intact nuclei. The ability of added DNA to reverse the enhancement of NM

phosphorylation by H1 suggests that this modulation is regional and dependent on the presence of free histone H1. It has been suggested that free H1 does exist transiently [Louters and Chalkley, 1985; Rasmussen and Garen, 1993] and that it might have a role in inhibiting the ability of calmodulin to activate its target enzymes [Rasmussen and Garen, 1993]. This effect is abolished by preincubation of histone H1 with DNA [Rasmussen and Garen, 1993].

Using purified proteins in vitro, it has been shown that the β-subunit of CK2 is the primary subunit interacting with polybasic peptides and that this interaction enables the catalytic α-subunit to recognize the otherwise unrecognizable proteins, such as ornithine decarboxylase and calmodulin, as substrates [Meggio et al., 1994; Nakajo et al., 1988; Flamigni et al., 1990]. In the present experiments, all histones enhanced the de novo phosphorylation of a set of proteins of similar apparent molecular weight, such as 180, 150, 110, and 28 kDa. These proteins were previously identified to be phosphorylated in the isolated NM as well as in intact cells [Tawfic and Ahmed, 1994a,b].

In the present study, the phosphorylation pattern of NM proteins was specific to the type of histone added and was accompanied by a differential phosphorylation pattern of both the α- and the β-subunits of CK2. The histones that induced a different pattern of CK2 subunit phosphorylation also demonstrated a different pattern of NM protein phosphorylation. The autophosphorylation of CK2 subunits as well as phosphorylation by $p34^{cdc2}$ kinase has been reported [Litchfield et al., 1992; Palen and Traugh, 1991] although the significance of these reactions remains unknown. Suggestions have been made that phosphorylation of β-subunit influences its substrate recognition and binding affinity [Lin et al., 1994; Filhol et al., 1992]. Taken together, our data provide further evidence relating the differential phosphorylation pattern of CK2 subunits (induced by different histones) to the selection of the set of NM proteins to be phosphorylated in a given state of cellular activity that requires histone displacement. This would suggest a subtle mode of regulation of protein kinase CK2.

The observed differential phosphorylation of CK2 subunits in the NM fraction under various conditions might be attributable to other kinases besides the autophosphorylation of the enzyme. Thus far, only $p34^{cdc2}$ kinase has been shown to phosphorylate CK2 at mitosis; however, we did not detect $p34^{cdc2}$ kinase activity toward its substrate histone H1. This might suggest either the absence of an NM-associated $p34^{cdc2}$ kinase, at least in resting hepatic cells, or its irreversible inactivation by the procedure used to isolate the NM fraction.

The above-described effects of histones on NM-associated CK2 activity and the consequent changes in NM protein phosphorylation may have important implications. For the transcriptional or replicational machineries to access DNA, histones, and specifically histone H1, might be displaced and even removed from DNA as suggested in many reports [Bresnick et al., 1992; Felsenfeld, 1992; Brooks and Jackson, 1994; Lewin, 1994]. These conditions should allow for a facilitated interaction between histones and NM proteins to maximally stimulate NM-associated CK2, thereby inducing modulations in phosphorylation of NM protein. The regional interaction between histones and NM might have global as well as local effects. The global effect would include the effect of NM protein phosphorylation on the tensional integrity of the tissue and consequent modulation of nuclear morphology and gene expression [Ingber, 1997]. This suggestion is supported by the notion that the phosphorylation cascade has a large mechanical component because of the induced dramatic changes in protein conformation, size and flexibility [Urry, 1992]. The local effect would include having a more active CK2 at the genomic regions where displacement of histones takes place. The biological significance of this effect might be inferred from several observations. First, topoisomerase II is a hallmark of the NM and is indispensable for removing any entanglement of DNA during replication or transcription. The activity of this enzyme is regulated through phosphorylation by protein kinase CK2 [Ackerman et al., 1988]. The phosphorylated C-terminal of topoisomerase II, which contains 10 phosphorylation sites for CK2, aids in chromosome segregation/condensation [Vassetzky et al., 1994]. Second, several enzymes that are essential for genomic activities and that may require histone displacement likewise are phosphorylated by CK2, such as RNA polymerases I and II, DNA ligase, and polymerase α-primase [Prignet et al., 1992; Zandomeni et al., 1986]. Third, CK2 can phosphorylate many oncoproteins and transcription factors, many of which are associated with the NM such as c-*myc, max, jun,* and p53 [Bousset et al., 1993]. In summary, the observed modification of NM protein phosphorylation by histones might be involved in the fine adjustment of

indispensable cellular functions, especially those that require histone displacement, namely, replication and transcription.

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