

## Chromosomal Protein HMGN1 Modulates the Phosphorylation of Serine 1 in Histone H2A

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**ABSTRACT:** Here we demonstrate that HMGN1, a nuclear protein that binds specifically to nucleosomes, modulates the level of histone H2A phosphorylation. In *Hmgn1*<sup>−/−</sup> cells, loss of HMGN1 elevates the steady-state levels of H2AS1ph throughout the cell cycle. In vitro, HMGN1 reduces the rate of Rsk2- and Msk1-mediated phosphorylation of nucleosomal, but not free, histone H2A. HMGN1 inhibits H2A phosphorylation by binding to nucleosomes since an HMGN mutant, which cannot bind to chromatin, does not inhibit the Rsk2- mediated H2A phosphorylation. HMGN2 also inhibits H2A phosphorylation, suggesting that the inhibition of H2A phosphorylation is not specific to only one member of this protein family. Thus, the present data add modifications of histone H2A to the list of histone modifications affected by HMGN proteins. It supports the suggestion that structural chromatin binding proteins can modify the whole profile of post-translational modifications of core histones.

Covalent post-translational modifications (PTMs<sup>1</sup>) of histones play a role in the epigenetic regulation of gene expression and are often associated with nuclear processes occurring in the context of chromatin. For example, a transient amplification of serine 10 phosphorylation of core histone H3 (H3S10ph) is a characteristic feature of mitosis and meiosis in vertebrates (1, 2). Likewise, serine 1 in histone H2A (H2AS1ph), one of the most abundant PTMs in histone H2A (3), is noticeably phosphorylated during mitosis in *Caenorhabditis elegans*, *Drosophila melanogaster*, and mammals (4). The timing and localization of H3S10ph and H2AS1ph during mitosis are similar, and both modifications peak in mitotic chromosomes, when the chromatin fiber is the most condensed (4). Although these modifications often serve as markers for mitotic chromosomes, it is not clear if they are in fact required for chromatin condensation since H3 phosphorylation is not essential for mitosis (2). Among various mitotic phosphorylation sites the functional redundancy may be one explanation for this finding. H3S10 and H2AS1 phosphorylation occurs not only in mitosis but also during other stages in the cell cycle; however in contrast to mitosis, in the other cell cycle stages the timing and location of these two histone phosphorylations differ (4). Interestingly, upon cellular stress, the effector kinases phosphorylate both histones H3 and H2A. Yet, while the phosphorylation of H3 leads to gene activation (5, 6) the phosphorylation of H2AS1 seems to inhibit transcription (7). Thus, as already amply documented, changes in PTM levels occur in response to wide and diverse intra- and extracellular signals.

The cellular levels of histone modification are not fixed; they are in a constant state of flux and reflect the equilibrium between the activities of the enzymes that modify and demodify specific sites. In addition, structural proteins such as histone H1 (8, 9) and HMGN (10, 11), which bind to nucleosomes and alter the compactness of the chromatin fiber, have also been shown to affect the levels of specific modifications in the tail of histone H3. HMGN proteins bind specifically to the 147 base pair nucleosomal core particle, the building block of the chromatin (12). The binding of these proteins to nucleosomes reduces the compaction of the chromatin fiber and alters the transcription, replication, and repair potential of chromatin templates (12–14). HMGN proteins have a modular structure and contact both the nucleosomal DNA and the histone through multiple interaction sites (15). A central, positively charged region contains the major HMGN–chromatin contacts (16, 17); however, additional sites of interactions have been identified. Thus, site-directed cross-linking of HMGN–nucleosome complexes indicates that the C-terminal region of HMGN1 is located near the amino terminal tail of histone H3 (17). Significantly, HMGN1 enhances the levels of acetylation in H3K14, reduces the level of phosphorylation of H3S10 and H3S28, and also changes the acetylation and methylation state of H3K9 (10). Taken together with the structural data, the results suggest that the close proximity of HMGN1 to the H3 tail in the HMGN1–nucleosome complex alters the ability of histone modifiers to access and modify residues in H3. So far it has not been studied in detail whether HMGN1 affects the modification levels in the tail of core histones other than H3.

Here we use *Hmgn1*<sup>−/−</sup> mouse embryonic fibroblasts (MEFs) and in vitro reconstitution experiments to demonstrate that HMGN1 modulates the phosphorylation of serine 1 in histone H2A. We find that loss of HMGN1 alters the

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<sup>1</sup> Abbreviations: PTMs, covalent post-translational modifications; MEFs, mouse embryonic fibroblasts (MEFs).

steady-state phosphorylation levels of H2AS1 and demonstrate that the binding of HMGN1 to nucleosomes inhibits the phosphorylation of H2A. Our findings extend the known range of histone modifications affected by HMGN1 and strengthen the possibility that HMGNs, and similar architectural chromatin binding proteins, are part of the mechanism that modulates the cellular levels of these epigenetic markers.

## EXPERIMENTAL DETAILS

**Materials.** Antibodies to phosphorylated S1-H2A/H4 were a kind gift from D. Allis (4). Kinases Msk1, Rsk2, and AuroraB were purchased from Upstate Cell Signalling Solutions (Msk1, expressed by baculovirus in Sf9 insect cells, Cat. No. 14-438, Lot No. 21948; Rsk2, or Rsk2/MAPKAP Kinase 1b (Y707), active, Cat. No. 14-428, and Aurora-B, active, Cat. No. 14-489). Another preparation of Msk1, expressed in Sf21 cells (Lot No. 24815), phosphorylates predominantly histone H3 (10). Nucleosome core particles, oligonucleosomes, histones, HMGNs, mutant HMGNs, and corresponding antibodies were prepared as described (18).

**Cell Culture, Protein Extraction, and Western Blotting.** Mouse *Hmgn1*<sup>-/-</sup> and *Hmgn1*<sup>+/+</sup> MEFs were generated and grown as described (14). The cells were grown to 70% confluency and treated with 50 ng/mL anisomycin. Mitotic cells were isolated by treating cultures with nocodazole (30 ng/mL) for 18–24 h followed by washing and adding 1×SDS-loading buffer with phosphatase inhibitors. To arrest cell cultures at the G1/S-phase border, *Hmgn1*<sup>+/+</sup> and *Hmgn1*<sup>-/-</sup> cells were treated with 2 mM thymidine (Sigma) for 16 h, released into fresh medium for 8 h, and blocked at the G1/S-phase border again by 0.4 mM mimosine (Sigma) for 16 h (19). Cells were released into fresh medium, and the samples were taken at 3 h intervals. Adherent cells were collected by 1×SDS-loading buffer with a cocktail of phosphatase inhibitors (Boehringer). Unattached mitotic cells were pelleted by gentle centrifugation, and dissolved in 1×SDS-loading buffer with protein phosphatase inhibitors. The samples were analyzed by Western immunoblotting using PVDF, secondary antibodies conjugated with horseradish peroxidase and ECL Plus kit (Amersham) as a substrate. For quantitative analysis Western blots were exposed to Biomax Light X-ray films (Kodak). The developed films have been scanned and analyzed by ImageQuant TL software.

**Labeling of Cultured Cells with <sup>32</sup>P<sub>i</sub>.** The cells were cultured to 50–60% confluency and incubated in phosphate-free DMEM for 30 min. The cells were labeled by <sup>32</sup>P<sub>i</sub> (ICN) with a final concentration of 0.5 mCi/mL medium for 1 h. Unincorporated isotope was removed by washing the cells with cold 1×PBS.

**In Vitro Phosphorylation Assay.** All assays were performed in 1×phosphorylation buffer (50 mM Tris-HCl, pH 8.0, 10% glycerol (v/v), 1 mM dithiothreitol (DTT), 0.1 mM EDTA, and phosphatase inhibitors from Calbiochem). Each 10 μL reaction contained nucleosome cores 0.2 mg/mL (or free histone H3, 0.05 mg/mL), 1 μCi of γ-<sup>32</sup>P-ATP, supplier-recommended activity of kinase, and various amounts of HMGN1 (added at specific molar ratio to core particle). The assay was performed at 37 °C for 30 min. The reactions were stopped by the addition of an equal volume of a SDS–gel

sample buffer (100 mM Tris-HCl, pH 6.8, 200 mM DTT, 2% SDS, 0.1% bromphenol blue, 20% glycerol), denatured for 5 min at 95 °C, and the proteins were resolved on 15% SDS–polyacrylamide gel. The gels were stained with Coomassie Brilliant Blue for estimation of protein quantities, scanned, and vacuum-dried. The radioactivity incorporated into the protein bands was visualized by a PhosphorImager and Storm scanner (Molecular Dynamics) and quantified with ImageQuant software.

**Phosphorylation Site Mapping.** To identify H2A peptide phosphorylated by Msk1, core particles have been <sup>32</sup>P-labeled. The histone H2A has been separated by reversed-phase HPLC and digested with endoproteinase Glu-C (Promega). The resulting peptides were resolved (Jupiter C18 column) and counted by BioScan QC-2000. The position of N-terminal peptide on the HPLC profile has been located by immunoblotting using anti-histone H2A antibody (Abcam), produced against a decapeptide from N-terminus of human H2A (SGRGKQGGA).

## RESULTS

We have shown before that HMGN1 modifies the rate of stress-induced phosphorylation of H3S10 and H3S28 (10). To obtain a broader view on the role of HMGN1 in the stress-induced phosphorylation of histones, we first examined the incorporation of <sup>32</sup>P into the core histones of *Hmgn1*<sup>+/+</sup> and *Hmgn1*<sup>-/-</sup> cells, before and after stimulation of quiescent cells by anisomycin (Figure 1A). We found that the levels of <sup>32</sup>P incorporated into H3 in anisomycin-stimulated *Hmgn1*<sup>-/-</sup> cells were significantly higher than those in anisomycin-stimulated *Hmgn1*<sup>+/+</sup> cells, a finding that is in full agreement with our previous conclusion that HMGN1 inhibits the phosphorylation of H3S10 and H3S28. We also noticed that the phosphorylation of non-histone proteins was similar in stimulated and nonstimulated *Hmgn1*<sup>+/+</sup> and *Hmgn1*<sup>-/-</sup> cells, indicating that general kinase activity was unaltered (Figure 1A). In addition, we noticed that loss of HMGN1 increased the anisomycin-stimulated incorporation of <sup>32</sup>P into H2A but decreased the incorporation of <sup>32</sup>P into H4 (Figure 1A).

Since anisomycin is known to stimulate the MAPK-signaling pathway cascade (20), we used recombinant Msk1 to label isolated nucleosomes with <sup>32</sup>P and resolved the nucleosomal histones on polyacrylamide gels containing either SDS or acid-urea-Triton X-100. Autoradiography of the gels revealed that in both systems only histones H3 and H2A were labeled by Msk1 (Figure 1B). Since we already studied histone H3, we proceeded to map the H2A residues phosphorylated by Msk1. <sup>32</sup>P-labeled H2A was purified from Msk1-treated nucleosomes, digested with Glu-C, and the resulting peptides separated by HPLC (Figure 1C). More than 80% of the <sup>32</sup>P counts comigrated with one peak, which was the only one that gave positive reactions with antibodies elicited against a decapeptide from the N-terminal tail of histone H2A (lower panel in Figure 1C). While these experiments were in progress, three manuscripts reported that histone H2A has a single major phosphorylation site (21), Msk1 specifically phosphorylates H2AS1 (7), and H2AS1 is phosphorylated in vitro (4). Based on all these observations we conclude that in our experiments Msk1 phosphorylates predominantly serine 1 in nucleosomal histone H2A.

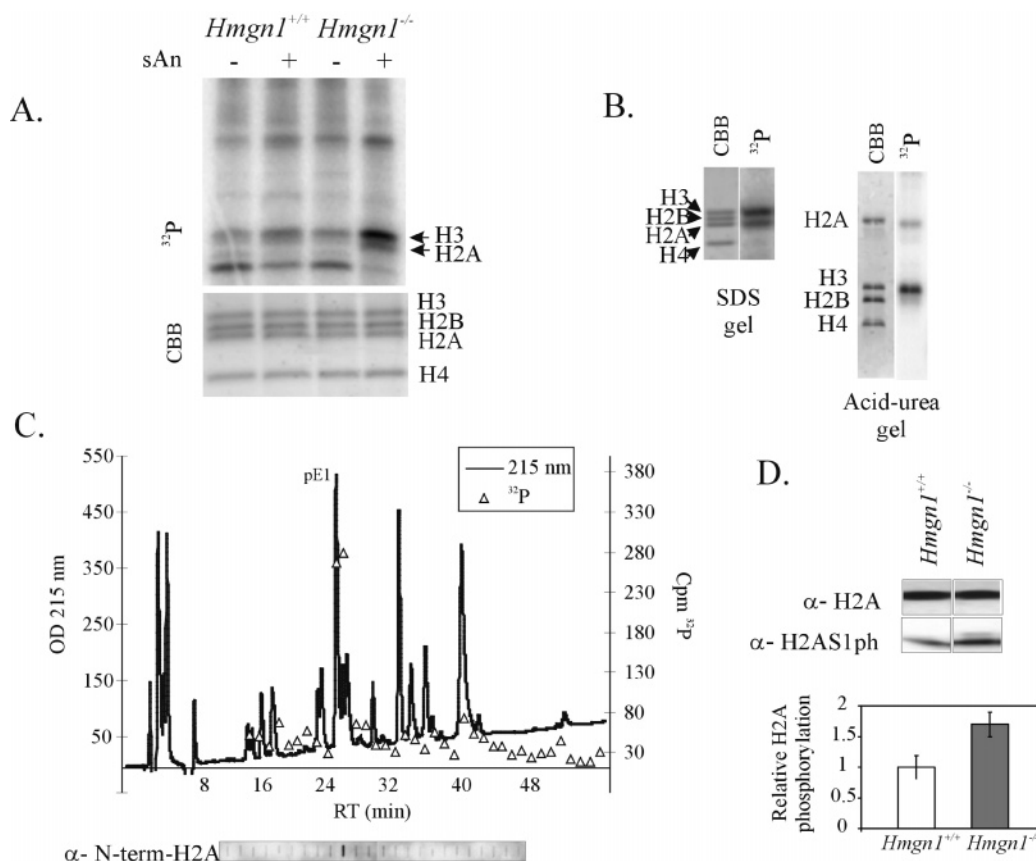


FIGURE 1: Elevated levels of H2A1ph in *Hmgn1*<sup>-/-</sup> cells. (A) Anisomycin-induced phosphorylation of histone H2A occurs in vivo and is affected by loss of HMGN1 protein. *Hmgn1*<sup>+/+</sup> and *Hmgn1*<sup>-/-</sup> mouse embryonic fibroblasts (MEFs) were pulse labeled by <sup>32</sup>P and treated with anisomycin. Nuclear proteins were visualized by Coomassie Brilliant Blue (CBB) staining of SDS-PAGE and PhosphoImaging. (B) Msk1 phosphorylates both histones H3 and H2A in nucleosomes. Chicken core particles were phosphorylated using <sup>32</sup>P-ATP and Msk1 in vitro and subjected to SDS-PAGE or acid-urea PAGE. Gels were stained by CBB and exposed to PhosphoImager. (C) Msk1 phosphorylates predominantly serine 1 of histone H2A. Labeled histone H2A was isolated and digested with Glu-C, and the resulting peptides were separated by reverse-phase HPLC (215 nm profile is shown). Fractions from HPLC were either counted by liquid scintillation and plotted (<sup>32</sup>P triangles) or immunoblotted with anti-N-terminal H2A peptide antibodies (panel below the elution profile). pE1 denotes the N-terminal peptide of histone H2A. (D) The level of H2AS1ph is higher in exponentially growing *Hmgn1*<sup>-/-</sup> cells. Proteins from *Hmgn1*<sup>-/-</sup> and *Hmgn1*<sup>+/+</sup> MEFs were analyzed by Western blotting using antibodies against histone H2A or against H2AS1ph. The bar graphs represent averages from three independent experiments. H2AS1 phosphorylation signals were normalized to the CBB signals in the corresponding samples.

To test whether in vivo the level of this modification is affected by HMGN1, we used antibodies specific to H2AS1ph (4) to compare histones extracted from growing *Hmgn1*<sup>-/-</sup> and *Hmgn1*<sup>+/+</sup> cells. The Western blotting clearly showed a higher level of H2AS1ph in growing *Hmgn1*<sup>-/-</sup> cells (Figure 1D, see also corresponding lanes in Figure 2A), as an indication that HMGN1 reduces the levels of H2AS1ph in vivo.

**Phosphorylation of H2AS1 in Response to Anisomycin Stimulation.** Since H2AS1 is a preferred phosphorylation site for Msk1 in vitro, and anisomycin stimulates the Msk signaling pathway, we used Western analysis with antibodies specific to H2AS1ph to examine the course of phosphorylation at H2AS1 at various times after anisomycin treatment of *Hmgn1*<sup>+/+</sup> or *Hmgn1*<sup>-/-</sup> mouse embryonic fibroblasts. In the quiescent cells, prior to anisomycin stimulation, the levels of H2AS1ph in *Hmgn1*<sup>+/+</sup> are approximately 2-fold lower than in the *Hmgn1*<sup>-/-</sup> cells, supporting the findings that the presence of HMGN1 lowers the level of this modification. Quantitative analysis of four separate measurements (Figure 2B) reveals that immediately after stimulation the levels of H2AS1ph decrease in both cell types, but with a more pronounced decrease in the *Hmgn1*<sup>-/-</sup> cells. Within 15 min,

the levels of H2AS1ph recovered and then gradually increased in both cells; however, in *Hmgn1*<sup>-/-</sup> MEFs, the stress-induced phosphorylation of H2AS1 peaked after 60 min, while in *Hmgn1*<sup>+/+</sup> MEFs it peaked later. The difference between *Hmgn1*<sup>+/+</sup> and *Hmgn1*<sup>-/-</sup> cells stays constant during anisomycin stimulation. These results suggest that loss of HMGN1 enhances the levels of H2AS1ph independently of anisomycin stimulation. We also note that the anisomycin induced changes in phosphorylation of H2AS1 were less pronounced than those previously observed for the phosphorylation of H3S10 and H3S28 (10, 22).

**Effect of HMGN1 on Cell Cycle Dependent Phosphorylation of H2AS1.** Synchronized human cells exhibit a biphasic profile of H2A1 phosphorylation: this modification peaks at early S-phase and in mitosis (4). We therefore compared the patterns of H2AS1ph during the cell cycle in *Hmgn1*<sup>+/+</sup> and *Hmgn1*<sup>-/-</sup> mouse embryonic fibroblast cells. The cells were treated sequentially with thymidine and mimosine (blocking the cells at the G1/S border), released into fresh medium to allow the cells to resume their cell cycle progression (19), and harvested at various time points post release. Coomassie blue staining and Western blotting with antibodies against histone H1 indicate equal loading of



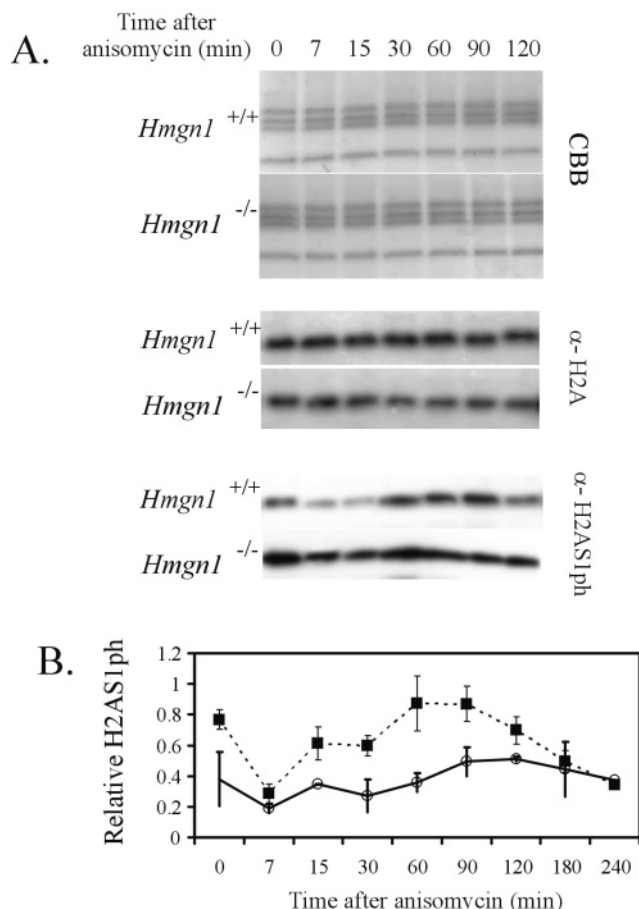


FIGURE 2: Loss of HMGN1 elevates the levels of H2A1ph throughout the anisomycin-induced stress response. (A) Extracts from *Hmgn1*<sup>+/+</sup> and *Hmgn1*<sup>-/-</sup> mouse embryonic fibroblasts, obtained at various times after stimulation of quiescent cells with anisomycin, were analyzed with either anti-H2A or anti-H2AS1ph antibodies (indicated on the right of Western blot panels). (B) Quantitative analysis of the levels of H2A1ph after anisomycin treatment. X-ray films of developed Western blots were scanned and quantified, and the values of the H2A1ph signal were normalized to those of the corresponding H2A signals. The standard deviation values were calculated from four independent measurements.

the gels and transfer to the blotting membranes (Figure 3A). By this analysis, mouse embryonic fibroblasts did not exhibit as strong a biphasic phosphorylation of H2AS1ph as previously reported for other cell types; however, throughout most of the cell cycle stages tested, loss of HMGN1 marginally increased the levels of H2AS1ph (Figure 3B), a finding that is in agreement with a role for HMGN1 in modulating the levels of this modification.

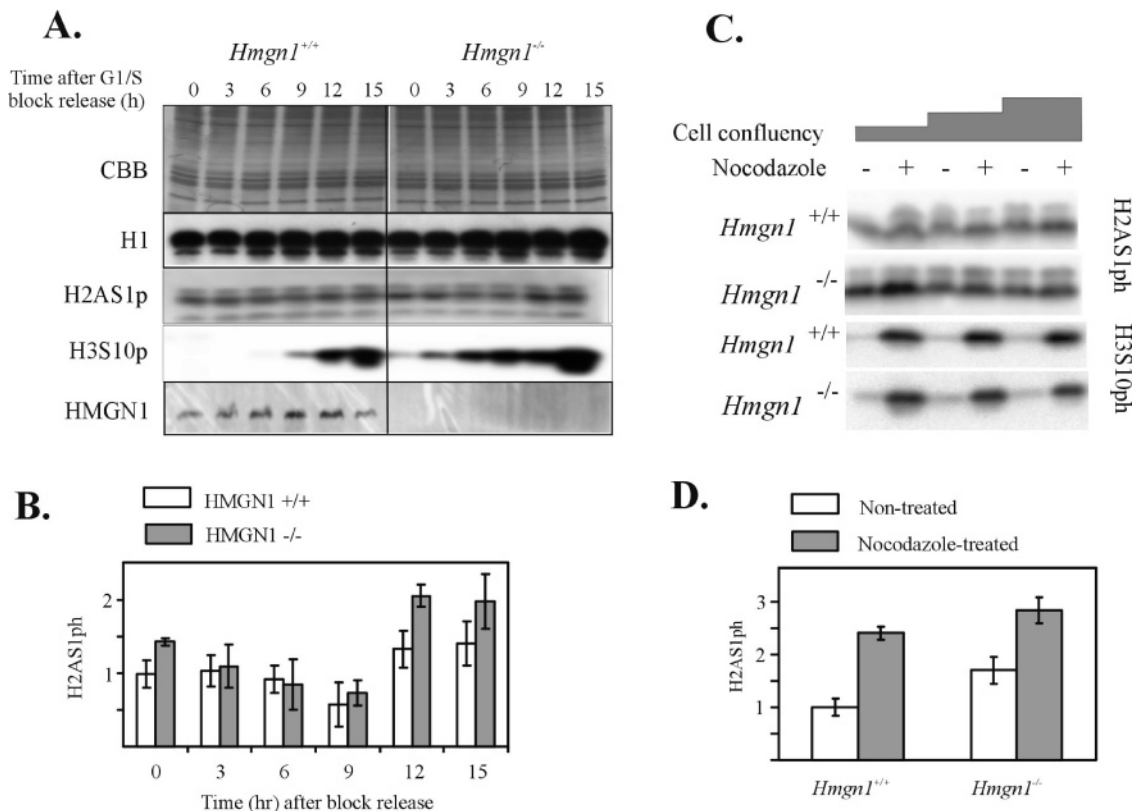
To further examine the role of HMGN1 in the mitotic phosphorylation of H2AS1, we examined the levels of this modification in nocodazole-arrested mitotic cell cultures, treated at 3 different confluence levels. Western analysis with anti-H3S10ph, a well-known mitotic marker, verified that, at all the confluence levels examined, the nocodazole treatment indeed produced a mitotic arrest (Figure 3C). Immunoblotting data indicate that the mitotic fluctuation in the levels of H2A1ph is less pronounced than that observed for H3S10. However, under all conditions tested, the levels of this modification were higher in *Hmgn1*<sup>-/-</sup> cells compared to *Hmgn1*<sup>+/+</sup> cells (Figure 3C, 3D).

**HMGN1 Inhibits Phosphorylation of Nucleosomal but Not of Free Histone H2A.** To understand the mechanism whereby HMGN1 inhibits the phosphorylation of H2A, we incubated purified nucleosome core particles (CP) with increasing amounts of HMGN1 and a constant amount of <sup>32</sup>P-ATP, and tested whether HMGN1 affects the activity of Rsk2 and Msk1. The levels of phosphorylation were determined by quantitative analysis of protein gels and their corresponding autoradiograms. HMGN1 inhibited H2A phosphorylation by both Rsk2 (Figure 4A) and Msk1 (Figure 4D), in a dose-dependent manner up to the point at which the nucleosome binding sites were saturated by HMGN1, and the maximum inhibition of phosphorylation coincided with the HMGN1 concentration that bound all the CPs specifically (Figure 4C, 4F). In these HMGN1–nucleosome complexes the level of phosphorylation was reduced by over 90% (Figure 4A, 4C, 4D, 4F). To verify further that histone H2AS1 phosphorylation is inhibited by HMGN1 only in nucleosomal context, we carried out these experiments using free histone H2A as a substrate. Both Rsk2 and Msk1 phosphorylated free histone H2A more efficiently than histone H2A within the nucleosome. HMGN1 protein did not inhibit the phosphorylation of free H2A (Figure 4B, 4C, 4E, 4F). Thus, the interaction of HMGN1 with nucleosomes inhibits the phosphorylation of H2A. Indeed, the double point mutant HMGN1 S20,24E, which does not bind specifically to nucleosomes (Figure 5A, top, see also ref 23), did not inhibit the phosphorylation of H2A (Figure 5A, 5C). Since we previously observed HMGN-variant specific effects on the modification of histone H3 (10, 11), we also tested whether HMGN2, another member of the HMGN protein family, inhibits the Rsk2-mediated phosphorylation of nucleosomal H2A. We found that as well as HMGN1 protein, HMGN2 inhibits efficiently this phosphorylation (Figure 5B, 5C), only if nucleosomes are used as the substrates. It suggests possible functional redundancy among HMGN variants in the ability to affect the levels of H2A phosphorylation.

## DISCUSSION

Previous studies demonstrated that HMGN1 modulates the levels of modification in the tail of histone H3 (10, 11). We now find that the interaction of HMGN1 with nucleosomes also impedes the rate of phosphorylation of serine 1 in the amino terminus of histone H2A. Thus, our present findings expand the known range of histone modifications affected by the interaction of HMGN proteins with nucleosomes. Several studies suggest that the interaction of linker histone H1 with chromatin also affects the levels of histone modification (8, 9). Taken together all of the data suggest that nucleosome binding proteins affect the ability of chromatin modifiers to access and modify their nucleosomal targets. The finding that architectural chromatin binding proteins modulate the levels of histone modifications suggests an additional mechanism that regulates the levels of these epigenetic markers.

**HMGNs Affect the Phosphorylation of H2A.** Several experiments linked the presence of HMGN1 protein to H2AS1 phosphorylation. First, the H2AS1ph levels in growing *Hmgn1*<sup>-/-</sup> cells were higher than in wild-type *Hmgn1*<sup>+/+</sup> cells. Second, loss of HMGN1 protein increased the steady-state level of H2AS1 phosphorylation in *Hmgn1*<sup>-/-</sup> before and during anisomycin-induced stress response and



**FIGURE 3:** Effect of HMGN1 on H2AS1ph during mitotic arrest and throughout the cell cycle. (A) After *Hmgn1*<sup>+/+</sup> and *Hmgn1*<sup>-/-</sup> cells were synchronized at the G1/S border, the growing medium was replenished with drug-free medium and the cells harvested at the indicated times. Whole-cell extracts were resolved by electrophoresis on a 15% SDS–polyacrylamide gel and immunoblotted with antibodies to H1, H2AS1ph, H3S10ph, or mouse HMGN1. (B) H2AS1 phosphorylation signals were plotted for *Hmgn1*<sup>+/+</sup> (white bars) and *Hmgn1*<sup>-/-</sup> (gray bars) as a function of time after G1/S block release. The bars represent mean values from four separate measurements, whereas error bars represent SD. (C) *Hmgn1*<sup>+/+</sup> and *Hmgn1*<sup>-/-</sup> MEFs, grown to different levels of confluence, were treated with nocodazole for 16 h and analyzed by Western blotting using antibodies against H2AS1ph and H3S10ph. (D) H2AS1 phosphorylation signals were normalized as above and plotted for nocodazole-treated (mitotic cells, gray bars) or nontreated (confluent cells, white bars) cells for the least-confluent *Hmgn1*<sup>+/+</sup> and *Hmgn1*<sup>-/-</sup> cells. The bars represent mean values from three separate measurements, whereas error bars represent SD.

during the cell cycle. Third, in vitro, HMGN1 inhibited the phosphorylation of nucleosomal H2A. The effect of HMGN1 is related to its interaction with chromatin because (1) HMGN1 S20,24E mutant, which does not bind to chromatin, did not inhibit phosphorylation; (2) the maximum inhibition of phosphorylation coincided with the maximum binding of HMGN1 to nucleosomes; and (3) HMGN1 inhibited the phosphorylation of nucleosomal but not free, purified H2A.

Several distinct mechanisms could account for the HMGN-mediated inhibition of H2A phosphorylation. One possibility is that the nucleosomal binding sites of HMGN partially overlap with those of the kinases thereby reducing the binding of the enzyme to its target. Since the interaction of HMGN with chromatin is highly dynamic (24), it is possible that dynamic competition between HMGN and kinases reduces the overall levels of H2A phosphorylation. Alternatively, the presence of HMGN on the nucleosome sterically hinders the ability of the enzyme to modify H2A. An additional possibility is that the binding of HMGN to nucleosomes induces structural changes in the nucleosome or in the histone tail that impede the ability of the enzymes to access and modify H2AS1. Indeed, kinetic analysis of the effect of HMGN1 on the PCAF-mediated acetylation of H3K14 indicated that HMGN1 affects both the apparent  $V_{\max}$  and the apparent  $K_m$  of the reaction, suggesting that the binding of HMGN1 to nucleosomes induces changes in the nucleosome itself, which ultimately modulate the ability of

the enzyme to modify the tail of core histones (11).

The major members of the HMGN family, HMGN1 and HMGN2, have similar effects on H2AS1 phosphorylation. Functional redundancy among members of the HMGN family could account for the relatively mild differences in H2AS1ph steady-state levels of *Hmgn1*<sup>+/+</sup> and *Hmgn1*<sup>-/-</sup> cells.

In two previous papers we demonstrated that HMGN1 modulates the modifications of histone H3 N-terminus (10, 11). Here we show that HMGN1 affects the rate of modification (phosphorylation) of the other histone, H2A. The structural difference between N-termini H2A and H3 is quite remarkable. First, histone H3 has the longest flexible amino terminus among core histones, whereas histone H2A has the shortest one. Second, H3 and H2A histone tails reach outside of the nucleosomal particle at opposite sites (25). Third, H3 histone is much more intensively modified in vivo than histone H2A (21). Despite the numerous differences between histones H2A and H3, the modifications of both histones are modulated by nucleosomal-bound HMGN1. It is tempting to speculate that HMGN family proteins are capable to modulate the modifications of the other histones as well.

*Architectural Chromatin Binding Proteins Affect the Levels of Histone Modifications.* Our finding that HMGN affects the phosphorylation of H2A extends the known range of histone modifications affected by HMGNs. Previous studies

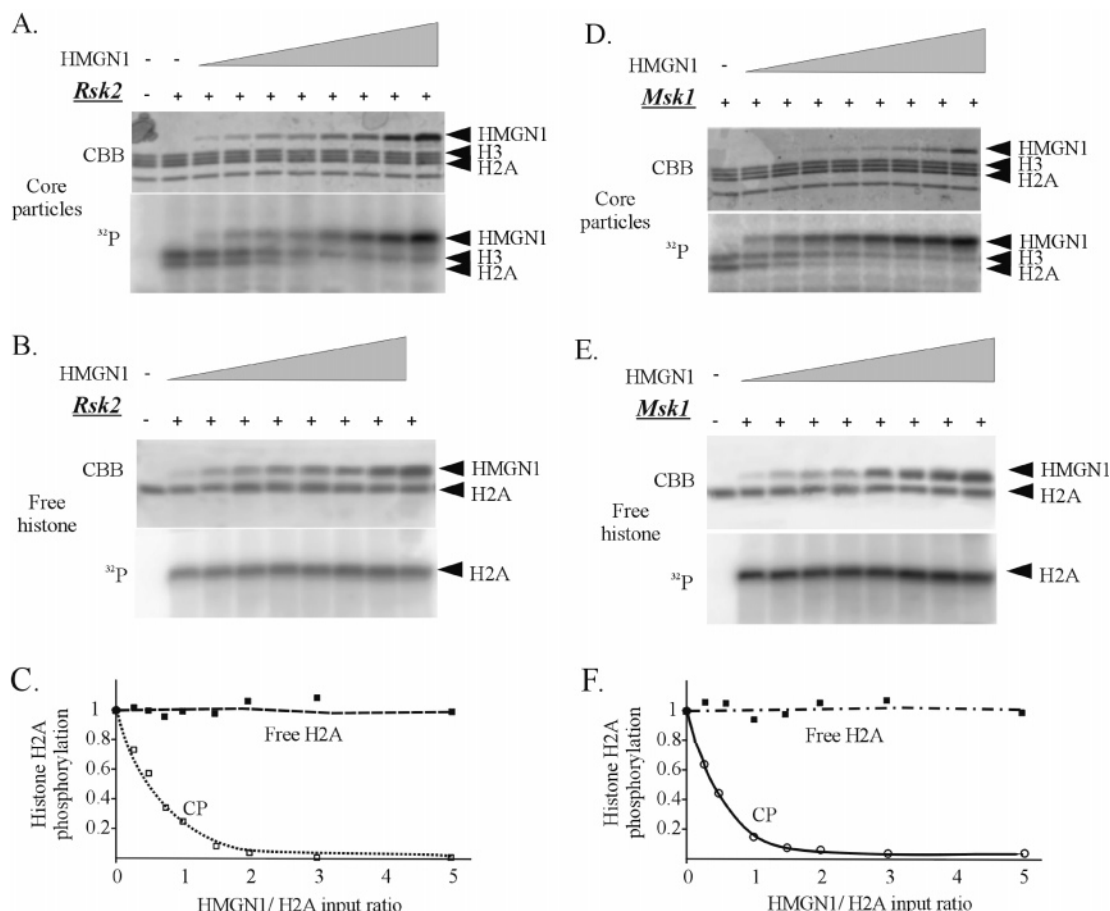


FIGURE 4: HMGN1 inhibits the phosphorylation of nucleosomal H2A. (A) HMGN1 inhibits the Rsk2-mediated phosphorylation of nucleosomal H2A. Reaction mixtures containing equal amounts of core particles (CP), but various amounts of HMGN1, were incubated with [ $\gamma$ - $^{32}\text{P}$ ] ATP and Rsk2, fractionated on 15% polyacrylamide SDS-containing gels. Shown are CBB-stained gels and corresponding autoradiograms. Note the dose-dependent, inverse correlation between HMGN1 input and H2A phosphorylation. (B) HMGN1 does not inhibit the Rsk2-mediated phosphorylation of purified H2A. Reactions as in panel A, except free, purified H2A was used as substrate. (C) Plot of the effect of HMGN1 on the Rsk2-mediated phosphorylation of free and nucleosomal H2A. (D) HMGN1 inhibits the Msk1-mediated phosphorylation of nucleosomal H2A. The experiment has been done exactly as described in panel A, except for Msk1 as a kinase. (E) HMGN1 does not inhibit the Msk1-mediated phosphorylation of purified H2A. (F) Plot of the effect of HMGN1 on the Msk1-mediated phosphorylation of free and nucleosomal H2A.

demonstrated that the interaction of HMGN affects several modifications in the tail of H3. Significantly, the effects are HMGN variant-specific since HMGN2 does not inhibit H3S10 phosphorylation and it increases H3K14 acetylation even more efficiently than HMGN1. In a recent study with HMGN1 and HMGN2 domain swap mutants the authors provided insights into the structural modules of HMGN1 and HMGN2 affecting the levels of PTMs (26). Taken together the data argues that the interaction of HMGNs with nucleosome affects the dynamic equilibrium between enzymes that continually modify and demodify the tails of the nucleosomal histones thereby changing the levels of PTM in chromatin.

In addition, early *in vitro* studies (8) and more recent experiments with H1 $^{-/-}$  mice (9) indicate that the histones H1, the most abundant family of structural chromatin-binding proteins, also affect the cellular levels of PTM. Conceivably both H1 and HMGN proteins could affect the pattern of PTMs in a gene-specific manner. The finding that both HMGNs and H1s affect the levels of chromatin modification strengthens the possibility that nucleosome binding proteins devoid of enzymatic activities are part of the mechanism that affects the levels of chromatin modifications. As such, these

studies point to an additional mechanism that regulates the levels of these important epigenetic markers.

## CONCLUSION

A series of recent publications have shown that chromosomal proteins such as HMGN and H1 (9) are capable of modifying the level of core histone PTMs in a specific manner with regard to HMGN. Several observations are of specific interest. First, HMGN1 inhibits the phosphorylation of H3-S10 (10) and facilitates the acetylation of H3-K14 (11), whereas several other modifications seem to be unaffected (10). Second, distinct modules in HMGN1 and HMGN2 affecting specific modifications have been identified (26). A major new finding described here is that HMGN1 (and, perhaps, HMGN2) affects PTMs of a core histone other than H3: histone H2A. The histone H2A N-terminus is located quite far from the histone H3 tail (25), and the fact that HMGN1 inhibits both H3 and H2A phosphorylation is consistent with the model in which the binding of HMGN to nucleosome core alters the accessibility of different histone tails to different modifying activities.



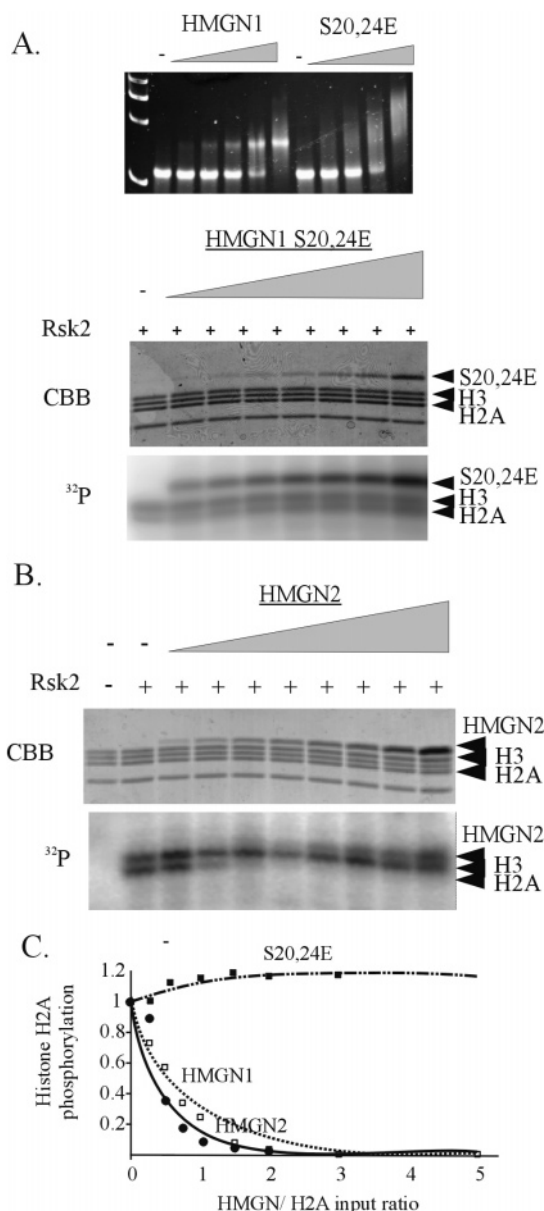


FIGURE 5: (A) HMGN1 inhibits the Rsk2-mediated phosphorylation of H2A by specific binding to nucleosome cores. Top: Mobility shift assays of purified chicken core particles (CP) with wild type HMGN1 and mutant the HMGN1S20,24 which does not bind to CP. Note that incubation of wild type HMGN1 (left) with CPs produced a distinct shift (CP+2HMGN1) while the HMGN1 S20,24E mutant (right) produces a nonspecific smear. In phosphorylation assay HMGN1 S20,24E mutant does not inhibit the Rsk2-mediated phosphorylation of nucleosomal H2A (two bottom panels). (B) HMGN2 inhibits the phosphorylation of nucleosomal H2A. Shown are CBB-stained gels and corresponding autoradiograms. (C) The graph depicts the effects of HMGN1 (data from Figure 4), HMGN1 S20,24E, and HMGN2 proteins on the Rsk2-mediated phosphorylation of nucleosomal H2A.

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