Chromosomal Protein HMGN1 Enhances the Heat Shock-induced Remodeling of Hsp70 Chromatin

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Galina I. Belova†, Yuri V. Postnikov‡, Takashi Furusawa‡, Yehudit Birger§, and Michael Bustin†‡

From the †Protein Section, Laboratory of Metabolism, NCI, National Institutes of Health, Bethesda, Maryland 20892 and §Cancer Research Center, Sheba Medical Center, Tel-Hashomer, Ramat Gan, Israel 52621

The nucleosome-binding protein HMGN1 affects the structure and function of chromatin; however, its role in regulating specific gene expression in living cells is not fully understood. Here we use embryonic fibroblasts from Hmgn1+/+ and Hmgn1−/− mice to examine the effect of HMGN1 on the heat shock-induced transcriptional activation of Hsp70, a well-characterized gene known to undergo a rapid chromatin re-structuring during transcriptional activation. We find that loss of HMGN1 decreases the levels of Hsp70 transcripts at the early stages of heat shock. HMGN1 enhances the rate of heat shock-induced changes in the Hsp70 chromatin but does not affect the chromatin structure before induction, an indication that it does not predispose the gene to rapid activation. Heat shock elevates the levels of H3K14 acetylation in the Hsp70 chromatin of wild type cells more efficiently than in the chromatin of Hmgn1−/− cells, whereas treatment with histone deacetylase inhibitors abrogates the effects of HMGN1 on the heat shock response. We suggest that HMGN1 enhances the rate of heat shock-induced H3K14 acetylation in the Hsp70 promoter, thereby enhancing the rate of chromatin remodeling and the subsequent transcription during the early rounds of Hsp70 activation when the gene is still associated with histones in a nucleosomal conformation.

The dynamic nature of the chromatin fiber plays a key role in genetic regulation, and therefore, it can be expected that architectural chromatin-binding proteins such as the linker histone H1 and the high mobility group (HMG) proteins (1–3) would affect transcription. Indeed, numerous studies demonstrated that both H1 and HMGs, which constitute a major superfamily of non-histones, affect cellular transcription levels (for reviews, see Refs. 2–9); however, the molecular mechanisms whereby these proteins modulate transcription in the context of chromatin are not fully understood. Thus, although in vitro studies on the function of H1 led to the general view that this protein family acts as general repressors of transcription (10, 11), subsequent studies with cells depleted of H1 (12, 13) suggested that in living organisms the function of these proteins is more complex (7), as decrease in H1 protein levels altered the nucleosomal repeat but led to only a moderate, gene-specific change in the cellular transcription profile (5, 13, 14). Likewise, studies on the cellular function and mechanism of action of HMGs suggest that these structural proteins affect multiple processes in the context of chromatin and that changes in the cellular levels of HMGs lead to both up and down-regulation of specific gene expression (9).

A possible explanation for the difficulties in unraveling the functions of architectural proteins comes from photobleaching experiments which revealed that in living cells H1 and HMGs continuously move throughout the nucleus, interact only transiently with chromatin, and compete for nucleosome binding sites (15–18). Thus, the cellular functions of H1 and HMGs may be interdependent, and therefore, elucidation of the biologically relevant role of a specific H1 or HMG variant in chromatin-related functions such as transcription is difficult to ascertain. Here we focus on the possible role of the nucleosomal-binding protein HMGN1 in the expression of a highly inducible gene in a biologically relevant context. We use cells derived from littermate Hmgn1−/− and Hmgn1+/+ mice to examine the role of HMGN1 in the expression of Hsp70, a well characterized gene inducible by heat shock (19–22). The heat shock response is an evolutionarily conserved protective mechanism operative in numerous cells (see references in Ref. 23).

HMGN1 is a major member of the HMGN protein family that is present in the nuclei of all mammalian cells. HMGNs bind specifically to nucleosomes (24), induce structural changes in chromatin, and have been shown to affect the levels of posttranslational modification in histone tails (25–28). In vitro studies indicated that HMGN1 enhances transcription in the context of chromatin (29–31), suggesting that it acts as a transcription coactivator. However, analysis of Hmgn1−/− cells indicates that loss of HMGN1 leads to both up and down-regulation of gene expression (32–34). Taken together, the available data suggest that HMGN1 does affect transcription from chromatin templates; however, the effect of HMGN1 on the chromatin structure and expression of a specific gene in a biologically relevant system has not yet been examined in detail.

Mice and cells lacking HMGN1 are viable; however, they are hypersensitive to various stresses (32, 33), and HMGN1 is modified during the stress-induced expression of immediate early genes (19, 25, 35). The highly inducible heat shock genes of Drosophila have been extensively used as a model to study chromatin changes during the transcriptional induction. In
mice, two closely related Hsp70 genes (shown schematically in Fig. 1B), termed Hsp70.3 and Hsp70.1 are located in the MHC region of chromosome 17 (36). They are nearly identical in coding sequence, differing in just two triplets. Although less well characterized than the Drosophila genes, both of the mouse Hsp70 genes are inducible and undergo significant changes in their chromatin in response to heat shock or chemical stresses (19, 20, 37, 38).

Here, we use embryonic fibroblasts (MEFs) from Hmgn1+/+ and Hmgn1−/− mice to study the transcription and chromatin structure of the heat shock-inducible mouse Hsp70 genes either in the presence or absence of HMGN1. The aim of the study is to examine the role of HMGN1 in the transcription and chromatin structure of a highly inducible gene in vivo. We find that loss of HMGN1 leads to alterations in the chromatin structure of the Hsp70 gene after, but not before heat shock induction. The Hsp70 promoter of heat-shocked Hmgn1−/− cells contains more H3 and is less sensitive to nuclease digestion then that of Hmgn1+/+ cells, suggesting that loss of HMGN1 reduces the rate of heat shock-induced chromatin remodeling. We find that heat shock elevates the levels of H3K14ac in the Hsp70 chromatin of wild type cells more efficiently than in the chromatin of Hmgn1−/− cells and that treatment with histone deacetylase (HDAC) inhibitors abrogates the effects of HMGN1 on the heat shock response. We conclude that HMGN1 enhances the rate of heat shock-induced acetylation of histones in the Hsp70 promoter, thereby enhancing the rate of chromatin remodeling and subsequent transcription during the early rounds of Hsp70 activation, when the gene is still associated with histones in a nucleosomal conformation.

**EXPERIMENTAL PROCEDURES**

**Materials**—Affinity pure antibodies to histones H3 and H1 and HMGN1 and HMGN2 were prepared as described (39). Anti-acetylhistone H3 (Lys-9) and anti-acetylhistone H3 (Lys-14) antibodies were purchased from Upstate Biotechnology, Inc., and anti-heat shock factor (HSF) antibodies were obtained from Santa Cruz Biotechnology, Inc.

**Cell Culture**—Primary mouse Hmgn1−/− and Hmgn1+/+ MEFs were first generated as described (33) and grown in Dulbecco’s modified Eagle’s medium, 10% fetal calf serum, 2 mM glutamine at 37 °C with 5% CO2. For survival studies, 20,000 cells were plated in 6-well plates with 2 ml of medium and grown for 24 h before a 30-min heat shock at 45 °C. After incubation the medium was replaced, the cells were cultured at 37 °C for various times, and viability was determined by trypan blue staining. Each point was done in triplicate.

**RNA Purification and cDNA Preparation**—RNA was isolated according to the manufacturer’s recommendation using Trysol® reagent (Invitrogen) and purified with RNeasy® kit (Qiagen). cDNA was prepared with iScript™ cDNA synthesis kit from Bio-Rad and used for quantitative real-time RT-PCR analysis.

**ChIP and Real-time Quantitative PCR**—ChIP experiments were performed according to the protocol recommended by Upstate with minor modifications. Each experiment was carried out with at least two different cell preparations; each DNA was analyzed by real-time quantitative PCR at least three times. In brief, cross-linked chromatin was lysed, sonicated (an average DNA fragment length of 400 bp), and subjected to immunoprecipitation with 8–10 μg of affinity-purified antibodies and 50 μl of protein A-agarose beads. The precipitated material was dissolved in 1% SDS, treated by protease K, phenol-extracted, and ethanol-precipitated. Real-time quantitative PCR was performed with SYBR Green Master Mix using the ABI PRISM 7900HT thermocycler. PCR primers to mouse genomic Hsp70 were either specific for single Hsp70.1 (primer pair 1 and 6) or for both Hsp70.3 and Hsp70.1 (primer pairs 2, 3, 4, and 5, see Fig. 1A) genes. Primers for β-actin and GAPDH genes were used for normalization. Sequence and exact gene positions of the primers are presented in supplemental Table I. Real-time PCR data were computed by SDS Version 2.0 software (40). -Fold differences (relative to control ChIP) were determined as 2((ΔΔCt sample) − (ΔΔCt control)), where ΔΔCt = ΔCt immunoprecipitated sample − ΔCt input and plotted. For every primer pair analyzed, each sample was quantitated at least in duplicate and from at least two immunoprecipitations. Specificity of PCR reactions were verified by both agarose gel electrophoresis and melting curve temperature profile.

**Chromatin Analysis**—Nuclei from Hmgn1+/+ and Hmgn1−/− MEFs before and after 7 min of heat shock were digested with 0.01, 0.1, and 1 units of micrococcal nuclease from Staphylococcus aureus (Sigma) for 5 min at 25 °C. The purified DNA was fractionated by electrophoresis in 1.5% agarose gel and transferred to a polyvinylidene difluoride membrane. Southern hybridizations with probes for various regions of the Hsp70 gene were performed. The probes were generated by random prime labeling of specific genomic regions which were generated by PCR amplification with specific primers (supplemental Table I). To improve the specificity of hybridization, high stringency conditions (0.1 × SSC (1 × SSC = 0.15 M NaCl and 0.015 M sodium citrate), 0.1% SDS at 68 °C) were used in the last washing step. Representative lanes from all four nuclei digests were quantified, and the average oligonucleosomal length was calculated using the formula \( L_n = \sum N_{pN} l_p(N) \), where \( L_n \) is the average oligonucleosomal length, \( N_p \) to \( N_n \) is the oligonucleosome size (i.e. mono, di), and \( p/N \) is the fraction of a particular oligonucleosome size in the total scan (the region covering mono- to tetra-nucleosomes was scanned) (32).

For DNase1 digests, MEFs before and after heat shock were harvested, and nuclei were isolated and resuspended in digestion buffer (60 mM KCl, 15 mM NaCl, 15 mM Tris- HCl, pH 7.4, 0.25 mM sucrose, 3 mM MgCl2, 0.5 mM dithiothreitol, 50% glycerol) at a concentration of 0.1 mg/ml. Digestions were carried out at 25 °C with 0.1, 0.5, 2.5, and 10 units of DNase I (Sigma Aldrich) per 1 μg of nuclei for 5 min. The samples were treated with protease K, and the DNA was recovered after extraction with phenol/chloroform/isopropanol and precipi-
The notion that cells lacking this protein are hypersensitive to heat shock is further supported by the finding that loss of HMGN1 results in decreased levels of Hsp70 transcripts. At short heat shock time points, the levels of transcripts were too low for accurate measurements. Transcripts were first reliably detectable after 15 min of heat shock, rapidly accumulating within the first 60 min and slowly decreasing afterward (Fig. 1C). We find that at the earliest stages of induction (15 min) the levels of Hsp70 mRNA in Hmgn1+/+ cells were twice that of Hmgn1−/− cells. However, after longer times of stimulation, as Hsp70 transcripts accumulate, the differences between the two cell types disappear, and levels of Hsp70 transcripts in Hmgn1+/+ cells were the same as that of Hmgn1−/− cells. The results suggest that HMGN1 protein may affect induction of transcription at early stages, whereas the genes are still associated with histones in a nucleosomal conformation. The decreased levels of Hsp70 transcripts in Hmgn1−/− fibroblasts could be a contributing factor to their heat shock hypersensitivity.

Loss of HMGN1 Alters the Heat Shock-dependent Remodeling of Hsp70 Promoter—To gain insights into the possible role of HMGN1 in the induction of the mouse Hsp70 gene, we first analyzed its organization in Hsp70 chromatin before and after heat shock. For the heat shock effects the analyses were done after 7 min of treatment so as to detect early events occurring before detectable transcript accumulation. For ChIP and quantitative PCR, we selected primer sets that amplified distinct regions of the genes ranging in length from 109 to 205 bp (Fig. 2 and supplemental Table I). Primer pairs 1 and 6 were specific to Hsp70.1 and Hsp70.3, whereas primers sets 2–5 amplified regions in which the genomic sequence of Hsp70.1 and Hsp70.3 was identical. Thus, primer pair 1 amplified a region located 60 bp upstream from the Hsp70.3 promoter, primer pair 2 amplified the promoter including TATA and CAAT boxes, primer pair 3 covered the start of transcription including the ATG, primer pair 4 amplified the center of the gene, primer pair 5 amplified the end of the transcribed region, primer 6 pair amplified a non-transcribed genomic region located immediately at the end of the Hsp70.3 gene, whereas primer pair 7 amplified a genomic region located in the center of the spacer separating Hsp70.3 and Hsp70.1 (see Fig. 1). In analyzing these experiments it is important to remember that the nuclear organization of most chromatin-binding proteins including HMGs and H1 is highly dynamic, and the proteins bind only temporarily to chromatin (15, 16, 18, 41). Thus, although most of the time most of the HMGN1 is bound to chromatin, at any specific chromatin site there is a continuous turnover of HMGN1 molecules. Therefore, the amount of chromatin immunoprecipitated reflects the residence time of HMGN1 at the particular chromatin site examined. Thus, the ChIP experiments provide information on the relative occupancy of HMGN1 at a particular chromatin locus at a specific time.

Before heat shock HMGN1 was evenly distributed throughout the gene as the relative amounts of the proteins did not vary among the various genomic regions (Fig. 2A). The relative amount of HMGN1 in the open reading frame of the gene was the same as that in its regulatory regions and in the surrounding, non-transcribed region of the gene. However, after 7 min of heat shock, the regions amplified by primer sets 2–4, corresponding respectively to the promoter, start of transcription, and the middle of the transcribed gene, were significantly depleted of HMGN1. The most significant depletion was at the
Loss of HMGN1 Reduces the Levels of Heat Shock-induced Alterations in Hsp70 Chromatin.—To test whether HMGN1 affects the heat shock-induced remodeling of Hsp70, we first probed the rate at which micrococcal nuclease digests the Hsp70 chromatin of Hmgn1+/+ and Hmgn1−/− MEFs, both before and after heat shock. The kinetics of micrococcal nuclease digestion of a particular genomic region, which can be evaluated by southern blotting, is an indication of the accessibility of the linker DNA to the enzyme, a general measure of chromatin “compactness” (45). We digested nuclei isolated from Hmgn1+/+ and Hmgn1−/− MEFs before and after heat shock, resolved the resulting bands on gels, and performed southern blots with specific probes for various regions of the Hsp70 gene already was low. However, histone H1 was significantly depleted from the transcribed portion of the gene but not from the non-transcribed regions amplified by primer sets 6 and 7. Although the binding of H1 to chromatin is significantly stronger than that of HMGN1, its nuclear organization is dynamic, and its interaction with chromatin is transient, just like many other nuclear proteins (18). The results indicate that heat shock changes the dynamic interaction of both HMGN1 and of histone H1 with Hsp70, most likely reflecting heat shock-induced local changes in the chromatin structure of this gene.

Indeed, ChIP analysis with anti-H3 antibody revealed that heat shock induces significant changes in the structure of the Hsp70 chromatin in both Hmgn1−/− and Hmgn1+/+ MEFs (Fig. 2C). Before heat stress, histone H3 was distributed evenly along the genes and surrounding areas, except in the promoter region amplified with primer set 2, where we repeatedly noticed a depletion of H3. In this respect the
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**FIGURE 3.** Decreased heat shock-induced remodeling of Hsp70 chromatin in cells lacking HMGN1, detected by micrococcal nuclease. A, Southern blot analysis of micrococcal nuclease digests of Hmgn1+/+ and Hmgn1−/− MEFs before and after heat shock. The bottom of the blots depicting the region containing mono to tetranucleosomes is shown. The position of the mono, di, and trinucleosomes is shown on the left (N1–N3). The probes used for hybridization are shown on the right. GAPDH and actin serve as controls indicating equal digestion of total DNA. B, scans of the mono and di nucleosomes region of the Southern blot are indicated. Note the heat shock-induced increase in the relative amount of the mononucleosome band in the promoter of the Hsp70 gene in Hmgn1+/+ as compared with Hmgn1−/− MEFs. Arrows point to the position of the mono and dinucleosomes (N1 and N2 respectively). C, the average length of the nucleosomal repeat calculated from scans of the Southern blot shown in panel A. Calculations of the average oligonucleosomal repeat are described under “Experimental Procedures.”

(A) Heat shock. (B) Hybridization signal. (C) Average nucleosomal length.

Actin and GAPDH served as controls for non-inducible genes. We then scanned the region containing mono to tetranucleosomes (boxed region in Fig. 3A) from chromatin digested under identical conditions and calculated the average length of the oligonucleosome of a specific genomic region of Hsp70 (Fig. 3C). The average length of the oligonucleosome is inversely proportional to the rate of micrococcal nuclease digestion since fast digestion will produce a larger number of short oligonucleosomes than slow digestion and, therefore, determination of the average oligonucleosome length in the digest is an indication of the rate of digestion and an estimate of chromatin “compaction.”

Before heat shock, the rate of micrococcal nucleosome digestion of the promoter regions (amplified by primer 2) in Hmgn1−/− MEFs was the same as in Hmgn1+/+ MEFs as evident from the relative amounts of mono and dinucleosomes present in the digest (left panel in Fig. 3B). In both cell types the average oligonucleosome length in the micrococcal nuclease digest of this region was 2.0 (Fig. 3C). We note, however, that this region is more sensitive to micrococcal nuclease digestion than the surrounding regions amplified by primers 1 and 5 or that of GAPDH and actin even before heat shock, an indication that the promoter of this gene is poised for transcription. After 7 min of heat shock, the rate of digestion of the Hsp70 promoter region (amplified by primers 2) increased significantly, and the average oligonucleosomal length was significantly smaller than before heat shock (Fig. 3C). Significantly, the average oligonucleosomal length of the promoter region in Hmgn1−/− cells (1.74 nucleosomes) was longer than that of Hmgn1+/+ cells (1.55 nucleosomes), an indication that loss of HMGN1 decreases the rate at which micrococcal nuclease digested this genomic region. This decrease rate of digestion is in full agreement with the ChIP data, which indicated a higher H3 occupancy in the Hsp70 promoter of cells lacking HMGN1 (Fig. 2C).

Quantitative PCR analysis of DNase I digested Hmgn1−/− and Hmgn1+/+ MEFs nuclei verified further that the Hsp70 gene is more accessible to this enzyme than both GAPDH and actin, especially after heat shock (Fig. 4A). The most prominent difference between wild type MEFs and MEFs lacking HMGN1 was the rate of digestion of the promoter regions (amplified by primer 2) after heat shock induc-
remodeling of Hsp70 chromatin, we first tested the levels of HSF bound to this region in heat-shocked Hmgn1−/− and Hmgn1+/+ MEFs. ChIP analysis failed to detect significant differences between the cells (Fig. 5A), an indication that HMGN1 does not have a major effect on the binding of HSF to its target and that the HMGN1-dependent differences in Hsp70 chromatin remodeling are not due to altered binding of HSF.

Alterations in the levels of posttranslational modification in the histones tails is known to be a key mechanism involved in chromatin remodeling during gene activation (46–48). Because HMGN1 affects the levels of H3K14ac (26, 28), a modification associated with transcription activation (49), we examined the levels of this modification before and after heat shock in Hmgn1−/− and Hmgn1+/+ MEFs. ChIP analysis reveals that in both cell types, heat shock induced a 2-fold increase in the level of H3K14ac in the genomic region 400 bp upstream of the Hsp70 gene and in the promoter region (Fig. 5B). In fact, the relative increase in the level of acetylation of H3K14 in Hsp70 chromatin, we chose to study the heat shock induction of the well characterized Hsp70 gene. We found that in Hmgn1−/− cells the levels of Hsp70 transcripts are lower at early, but not late stages of the heat shock induction. These results suggest that HMGN1 enhances Hsp70 transcription, whereas the gene is still associated with histones, a finding consistent with previous results indicating that HMGN1 affects transcription from chromatin but not “naked” DNA templates (for reviewed, see Ref. 24).

It has been suggested that HMGNs affects transcription by altering the chromatin structure of genes that are either active

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remodeling of Hsp70 chromatin is approximately twice that present in wild type Hmgn1+/+ cells. It, therefore, follows that the level of acetylation of the H3K14 residues in the heat-shocked promoter of the Hmgn1−/− cells is approximately twice that of Hmgn1+/+ cells (Fig. 5C, primer pair 2). In fact, the acetylation level of H3K14 in Hmgn1−/− cells is somewhat higher even in the transcribed portion of the heat-shocked Hsp70 (especially primer pair 3 in Fig. 5C). Taken together with our previously published results that HMGN1 affects H3K14 acetylation (26, 28), all of the data are consistent with the possibility that loss of HMGN1 decreases the rate of heat shock-induced H3K14 acetylation, thereby leading to a reduced rate of chromatin remodeling and a decreased amount of Hsp70 transcripts.

To further examine the possibility that HMGN1 affects the rate of Hsp70 activation by enhancing H3 acetylation, we tested whether elevation of histone acetylation levels before heat shock would abrogate the effect of HMGN1 on the activation of Hsp70. MEFs derived from littermate Hmgn1−/− and Hmgn1+/+ mice were incubated with HDAC inhibitors and heat-shocked for 15 min, and the levels of Hsp70 transcripts were quantified. In the absence of HDAC inhibitors, the levels of Hsp70 transcripts in RNA extracted from heat-shocked Hmgn1−/− cells was ~60% that detected in Hmgn1+/+ cells (Fig. 5C, and see also Fig. 1C). Trichostatin A treatments before heat shock abolishes the difference between Hmgn1+/+ and Hmgn1−/− cells (Fig. 5, C and D), further evidence that the effect of HMGN1 is mediated through histone acetylation. The results suggest that HMGN1 enhances the initial rounds of Hsp70 transcription by elevating the levels of histone H3 acetylation.

DISCUSSION

Our studies with Hmgn1−/− and Hmgn1+/+ cells reveal that in living cells, HMGN1 enhances the rate of heat shock-induced chromatin remodeling in the Hsp70 promoter, thereby leading to an increase in the levels of Hsp70 transcripts during the early stages of heat shock induction.

The role of chromatin architectural proteins such as HMGN in chromatin function including transcription is not still fully understood. The availability of Hmgn1−/− cells, in which the HMGN gene has been deleted, provides an opportunity to examine the role of HMGN1 in transcription in living cells. Comparison with Hmgn1+/+ cells derived from littermate mice ensures that the effects seen are indeed due to lack of HMGN1 rather to “off target” effects which can occur when cells are manipulated to either reduce or enhance the expression of a specific gene. To test for a possible role of HMGN1 in transcription from “native” cellular chromatin, we chose to study the heat shock induction of the well characterized Hsp70 gene. We found that in Hmgn1−/− cells the levels of Hsp70 transcripts are lower at early, but not late stages of the heat shock induction. These results suggest that HMGN1 enhances Hsp70 transcription, whereas the gene is still associated with histones, a finding consistent with previous results indicating that HMGN1 affects transcription from chromatin but not “naked” DNA templates (for reviewed, see Ref. 24).
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FIGURE 5. Heat shock-induced alteration in the H3K14 acetylation levels in the Hsp70 promoter. A, HMGN1 does not affect the recruitment of HSF to the Hsp70 gene promoter. Shown are results from ChIP-PCR analysis using anti-HSF antibodies and primers to Hsp70 promoter and to GAPDH gene which served as the control. B, heat shock-induced acetylation of Hsp70 promoter. Shown are levels of H3K14ac on the Hsp70 gene chromatin in Hmgn1+/+ and Hmgn1−/− cells before and after the heat shock. C, loss of HMGN1 decreases the acetylation level of H3K14 in the heat-shocked Hsp70 promoter. Shown are the H3K14ac/H3-specific ratios calculated by dividing the levels of acetylation by the levels of histone H3. D, Hsp70 gene induction in presence of HDAC inhibitor in Hmgn1+/+ and Hmgn1−/− cells. Cells grown either without or with Trichostatin A were shifted from 37 to 42 °C for 15 min, and the level of Hsp70 transcripts was visualized by ethidium bromide staining. The images were scanned and quantified. E, as in D, but the levels of heat shock-induced Hsp70 transcripts were analyzed by real-time reverse transcription-PCR. HS, heat shock.

in, or poised for transcription (24). Thus, one possible explanation for the HMGN1-dependent effects on Hsp70 activation is that loss of HMGN alters the structure of the Hsp70 chromatin even before heat shock. Early studies suggested that the chromatin of actively transcribed globin gene is depleted of H1 and enriched in HMGNs (50). Likewise, in an in vitro chromatin assembly system HMGN1 alleviates H1-mediated inhibition of transcription from SV40 promoters (29). These findings correlate well with more recent findings indicating that in living cells HMGN proteins compete with H1 for chromatin binding sites (43). Thus, HMGN-dependent alteration in the interaction of H1 with Hsp70 chromatin could be one possible mechanism explaining the ability of HMGN1 to enhance the heat shock-induced transcription.

To test for possible effects of HMGN1 on chromatin structure, we examined the nuclelease sensitivity of the Hsp70 gene in Hmgn1−/− and Hmgn1+/+ cells both before and after heat shock induction. In both cell types the promoter region (amplified by primer pair 2) was the most sensitive to nuclease digestion even before heat shock, a finding that is consistent with previous reports that the promoter of this gene is poised for transcription. In these digestions we did not find any significant differences between the Hmgn1−/− and Hmgn1+/+ cells, suggesting that HMGN1 does not alter the chromatin structure of the uninduced gene and does not predispose it to enhanced activation. Conversely, we detect significant HMGN1-dependent changes in the chromatin structure of the induced Hsp70 gene, especially in the promoter region. Induction of Hsp70 transcription by heat shock results in significant changes in the chromatin structure which are reflected by increased rate of digestion by both micrococcal nuclease and DNase1. We find that the induced Hsp70 promoter of Hmgn1+/+ cells is more sensitive to digestion than that of Hmgn1−/− cells (Figs. 3 and 4), suggesting that HMGN1 enhances the rate of heat shock-induced changes in the Hsp70 chromatin. Indeed, ChIP analysis reveal that within 7 min of induction the amount of histone H3 present in the Hsp70 promoter of Hmgn1−/− cells is twice that remaining in the promoter of Hmgn1+/+ cells (arrow in Fig. 2C). The decreased nuclease sensitivity and the decrease in the rate at which H3 is evicted from the Hsp70 promoter of the Hmgn1−/− cells correlates well with the decrease in the levels of Hsp70 transcripts in these cells. Thus, HMGN1 enhances the rate at which nucleosomes are evicted from the heat-shocked promoter, thereby providing an explanation for the increase in transcription and accumulation of Hsp70 transcripts in wild type Hmgn1+/+ cells.

The altered rate of nucleosome eviction could be due to changes in H1 chromatin occupancy, since HMGN1 competes with histone H1 for chromatin binding sites (17, 43) and since H1 affects chromatin structure and inhibits chromatin remodeling (5, 51, 52). We, therefore, tested the occupancy of H1 on the Hsp70 gene before and after heat shock. Our ChIP analysis reveal that histone H1 is significantly depleted from the promoter area of Hsp70 gene already before heat shock (Fig. 2B), providing additional support for earlier findings that this gene is poised for transcription before gene induction. Within a few minutes of heat shock induction, H1 is depleted also from the transcribed portion of the gene, a finding that is in agreement with previous observations on the heat shock induction in Drosophila cells (42). Repeated analysis of H1 occupancy on the
Hsp70 gene in Hmgn1−/− and Hmgn1+/+ cells before and after heat shock failed to detect any effect of HMGN1 on the interaction of histone H1 with Hsp70 chromatin. Thus, HMGN1 does not play a major role in the removal of the linker histone H1 from the transcribed Hsp70 chromatin.

The first step in the heat shock-induced activation of Hsp70 is the binding of the HSF to the Hsp70 promoter, a step that subsequently leads to changes in the levels of histone modifications. Although the HSF binding step is conserved in all organisms investigated, the type of histone modification varies among species (19). Our ChIP analyses reveal that loss of HMGN1 does not affect the recruitment of HSF to the Hsp70 promoter (Fig. 5A). These results suggested that HMGN affects a process downstream from HSF binding, a possibility supported by previous findings that HMGN proteins modulate the levels of certain histone modification, including the acetylation of H3K14 (26, 28). Indeed, our ChIP analyses indicate that heat shock treatment elevates the level of this modification 2-fold, leading to the initial impression that this increase is not affected by HMGN1. However, the H3K14ac/H3-specific ratio is a more precise indicator of local acetylation levels of certain histone modification, including the acetylation of H3K14 (26, 28). Indeed, our ChIP analyses indicate that heat shock treatment elevates the level of this modification 2-fold, leading to the initial impression that this increase is not affected by HMGN1. However, the H3K14ac/H3-specific ratio is a more precise indicator of local acetylation levels of histone H3 lysine 14. Our ChIP with anti-H3 clearly indicated that the amount of H3 in Hsp70 promoter of heat shocked is twice that present in wild type Hmgn1−/− cells. It, therefore, follows that the level of H3K14 acetylation in the Hsp70 promoter of Hmgn1−/− cells is twice that of Hmgn1−/− cells (Fig. 5C). Thus, loss of HMGN1 reduced the level of H3K14 acetylation, a finding that is in full agreement with previous results demonstrating that HMGN1 enhances H3K14 acetylation (26, 28). Our analyses of HDAC-treated cells indicate that the HMGN1-mediated change in histone acetylation is functionally significant since preincubation with HDAC inhibitors before heat shock abrogated the HMGN1 effects; the level of Hsp70 transcripts in Hmgn1−/− cells was the same as those in Hmgn1+/+ cells (Fig. 5).

Our findings provide additional insights into the cellular function and mechanism of action of HMGN proteins. Although HMGN1 affects the onset of Hsp70 transcription, it is not a heat shock-specific factor since the Hsp gene is robustly activated also in cells lacking HMGN1. Likely functional redundancy with other HMGN variants may dampen the deleterious effect of lack of HMGN1. It is relevant that HMGN1 also optimizes the cellular response to both UV and x-ray damage but only in the context of chromatin (32, 33). Our study suggests that in living cells the effects of HMGN1 on Hsp70 transcription are mediated through its ability to affect the rate of histone modifications. Taken together, the results suggest that the chromatin-modifying activities of HMGN1 optimize chromatin alterations necessary for the orderly progression of DNA-related activities such as transcription or repair. Interestingly, depletion of H1 also leads to alteration in chromatin modification and impairs the rate of DNA repair (53, 54). The emerging picture suggests that chromatin architectural proteins affect the levels of chromatin modifications, thereby participating in epigenetic regulation of gene expression and affecting the cellular phenotype.

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