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Alleviation of Histone H1-Mediated Transcriptional Repression and Chromatin Compaction by the Acidic Activation Region in Chromosomal Protein HMG-14

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Histone H1 promotes the generation of a condensed, transcriptionally inactive, higher-order chromatin structure. Consequently, histone H1 activity must be antagonized in order to convert chromatin to a transcriptionally competent, more extended structure. Using simian virus 40 minichromosomes as a model system, we now demonstrate that the nonhistone chromosomal protein HMG-14, which is known to preferentially associate with active chromatin, completely alleviates histone H1-mediated inhibition of transcription by RNA polymerase II. HMG-14 also partially disrupts histone H1-dependent compaction of chromatin. Both the transcriptional enhancement and chromatin-unfolding activities of HMG-14 are mediated through its acidic, C-terminal region. Strikingly, transcriptional and structural activities of HMG-14 are maintained upon replacement of the C-terminal fragment by acidic regions from either GAL4 or HMG-2. These data support the model that the acidic C terminus of HMG-14 is involved in unfolding higher-order chromatin structure to facilitate transcriptional activation of mammalian genes.

In mammalian cells, genomic DNA is highly condensed, being organized in the nucleoprotein complex constituting chromatin (65, 70). The packaging of genomic DNA into chromatin can inhibit gene expression in multiple ways: restricting the access of transcription factors to their promoter elements, blocking assembly and initiation by the general transcriptional machinery, and inhibiting elongation by the RNA polymerase. As a corollary, activation of transcription requires remodeling of the chromatin structure of the gene in order to relieve the repressive effects of chromatin on transcription (34, 38, 45, 71, 72).

The building block of chromatin is the nucleosome, consisting of DNA wrapped twice around a histone octamer comprising the core histones H2A, H2B, H3, and H4. A linker histone, generally histone H1, interacts with DNA at several sites simultaneously (3, 51, 55, 58): (i) with DNA at or close to the entry and exit points of the nucleosome, (ii) with nucleosomal DNA over the dyad axis, after one wrap around the octamer, and (iii) with linker DNA between adjacent nucleosomal core particles. Within the interphase nucleus of higher eukaryotic cells, the linear array of nucleosomes is folded into a higher-order structure, called the 30-nm chromatin fiber (65, 70). Histone H1 plays a key role in the formation of such a higher-order chromatin structure (4, 37, 52, 59, 60).

Biochemical and genetic studies have identified two distinct systems capable of remodeling the nucleosome structure for transcriptional activation. The multisubunit SWI/SNF complex, which is conserved from yeasts to humans (49, 69), can perturb the structure of a nucleosome in an ATP-dependent manner (15, 29, 35). This promotes the binding in vitro of either specific or general transcription factors, such as GAL4 and TBP, to their sites on nucleosomal DNA. A distinct nucleosome-remodeling factor (NURF), originally purified from *Drosophila* embryo extracts (63), is also capable of perturbing the structure of a mononucleosome in an ATP-dependent manner and of altering chromatin structure in concert with sequence-specific transcription factors.

Whereas SWI/SNF and NURF have been shown to locally alter the structure of nucleosomes in promoter regions, little is known about factors that remodel, or decompact, higher-order chromatin structure. In vitro experiments have demonstrated that chromatin compaction inhibits both initiation and elongation of transcription (27, 32). In cells, transcriptionally competent or active genes exhibit a more open, accessible chromatin structure than the bulk, transcriptionally inactive chromatin. This is exemplified by enhanced overall sensitivity of active chromatin to nucleases and by slower sedimentation of active chromatin fragments through a sucrose gradient (21, 31, 33, 57, 68). Strikingly, the establishment of an extended chromatin structure invariably precedes expression of genes during cell growth and development (21, 57), suggesting that this alteration in the higher-order chromatin structure is an early, essential step in transcriptional activation of genes.

The two closely related, highly conserved nucleosome-binding proteins HMG-14 and HMG-17 have long been suggested to play an important role in the generation of transcriptionally active chromatin (11, 13). It has been hypothesized that HMG-17 or HMG-14 could alter the chromatin structure of particular genes by accessing the newly assembled chromatin during the process of cellular DNA replication. In support of this hypothesis, chromatin reconstituted in vitro, which is generally transcriptionally repressed, can be altered by addition of HMG-17 or HMG-14 during, but not after chromatin assembly, to facilitate transcription by both RNA polymerase II (pol II) and pol III (17, 46, 61).

We recently described the activation of pol II transcription by HMG-14 or HMG-17 by using an alternative native, in vivo-assembled chromatin system, simian virus 40 (SV40) minichromosomes (MCs). In this system, the binding of HMG-14 to

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transcriptionally competent, preassembled nucleosomes stimulated elongation by pol II (19). Unlike other systems using chromatin reconstituted in vitro, the initiation of transcription in this system is unaffected by HMG-14, apparently due to the high transcriptional competency of the nucleosome-free SV40 late promoter. These data supported a novel, DNA replication-independent mechanism for activation of chromatin templates by HMG-14 or HMG-17, suggesting that HMG-14 and HMG-17 might also be involved in the rapid induction of gene expression in mammalian cells.

In this study, we have investigated the mechanism by which HMG-14 stimulates transcription from preassembled chromatin templates. We demonstrate that HMG-14 increases the rate of transcriptional elongation by pol II. Conversely, histone H1, which compacts chromatin, decreases the rate of transcriptional elongation. In fact, HMG-14 specifically alleviates both inhibition of transcriptional elongation and compaction of chromatin structure mediated by histone H1. Both activities require the negatively charged C-terminal region of HMG-14. Fusion proteins in which the acidic C-terminal region of HMG-14 is replaced either by the acidic activation domain I of GAL4 or by the acidic C-terminal tail of HMG-2 also stimulate transcription and decompact SV40 MCs, demonstrating that negative charge is a critical feature of the activation domain of HMG-14. Taken together, our data link structural and transcriptional features of chromatin and provide a molecular model for the generation of an active, unfolded chromatin structure, which may be amenable to rapid induction into a transcriptionally active state. Thus, HMG-14 and HMG-17 can be biochemically classified as chromatin-remodeling factors, but with structural implications extending beyond the single-nucleosome level.

MATERIALS AND METHODS

Isolation and manipulation of SV40 MCs. Native SV40 MCs were purified from crude SV40 MC extracts by 15 to 30% sucrose gradient sedimentation (7). Histone H1-depleted SV40 MCs (core-MCs) were prepared by sucrose gradient centrifugation of crude SV40 MC extract in the presence of 0.5 M NaCl. A portion of the core-MC preparation was incubated with pure histone H1 (Boehringer-Mannheim; input H1/nucleosome ratio [H1/n ratio] = 1.0), and the mixture was dialyzed overnight against a buffer containing 50 mM HEPES (pH 7.5) and 15% sucrose to reconstitute histone H1-containing SV40 MCs (core/H1-MCs), which were then purified by sucrose gradient sedimentation.

Analyses of SV40 MCs. For determination of the protein composition of SV40 MCs, proteins were extracted from MCs with 0.4 N H_2SO_4 and precipitated with 25% trichloroacetic acid prior to gel electrophoresis. The stained protein gel was scanned with an LKB Pharmacia laser densitometer to obtain histone H1 and H4 densities. From standard curves of increasing levels of pure histones H1 and H4, the H1/n molar ratios were determined. Micrococcal nuclease digestion of SV40 MCs was performed in 10 mM Tris-HCl (pH 7.5)–50 mM NaCl–1 mM $CaCl_2$–1.5 U of micrococcal nuclease (Worthington), with 3 μg of MCs (DNA content) at 37°C. Digestion was terminated with 10 mM EDTA. After phenol-chloroform extraction and ethanol precipitation, the DNA products were analyzed by 4% agarose gel (NuSieve 3:1; FMC) electrophoresis and ethidium bromide staining.

Construction, expression, and purification of HMG-14 and its mutant derivatives. DNA fragments coding for full-length HMG-14 and its deletion mutants were generated by PCR from pSVL14 (23) and were cloned into the *Bam*HI-*Kpn*I sites of the bacterial expression vector pQE-30 (Qiagen) to generate the plasmids pQE-HMG-14, pQE-HMG-14(12-99), pQE-HMG-14(1-73), and pQE-HMG-14(1-46). DNA fragments coding for the acidic activation domain I of GAL4 (residues 147 to 196) and the C-terminal 26 amino acids of HMG-2 (residues 184 to 209) were generated by PCR from pSG4 (a gift of Mark Ptashne, Harvard University) and pHis-HMG2 (a gift of Jaesang Kim and Phillip A. Sharp, Massachusetts Institute of Technology), respectively. The PCR products were fused to the 3′ end of a DNA coding sequence for HMG-14 (residues 1 to 73) through an *Eco*RI linker, and the resulting fusion sequences were then cloned into the *Bam*HI-*Kpn*I sites of pQE-30 to construct the plasmids pQE-HMG-14(1-73)/GAL4 and pQE-HMG-14(1-73)/HMG-2. A PCR-generated DNA fragment coding for the activation domain I of GAL4 was directly cloned into the *Bam*HI-*Kpn*I sites of pQE-30 to produce the plasmid pQE-GAL4AD1. The DNA sequences coding for HMG-14 (with a 3′ stop codon) and HMG-14(1-73) were also cloned into the *Bam*HI-*Eco*RI sites of pET-28a(+) (Novagen), resulting in plasmids pET-T7 · HMG-14 and pET-T7 · HMG-14(1-73)/C24. The latter plasmid would express a fusion protein with its C-terminal 24 amino acids (DFDLRRQACGRTRAPPPPPLRSGC) encoded by the polylinker region of pET-28a(+). All DNA coding sequences were verified by dideoxy sequencing.

Escherichia coli JM109 and BL21(DE3) were transformed with the pQE and the pET expression constructs, respectively. Expression was induced by addition of IPTG (isopropyl-β-D-thiogalactopyranoside). For purification of HMG-14 deletion mutants, the harvested bacteria were resuspended at 4°C in 5% perchloric acid and lysed by sonication. After a 1-h incubation and centrifugation of the lysates, the deletion mutants in the supernatants were precipitated by 25% trichloroacetic acid. The pellets were washed with cold acetone and resuspended in buffer A (50 mM NaH_2PO_4 [pH 8.0], 300 mM NaCl). Each protein solution was absorbed onto Ni-nitrilotriacetic acid (Ni-NTA) resin (Qiagen), which was subsequently washed with buffer A plus 30 mM imidazole. The HMG-14 derivatives were eluted with buffer A plus 100 mM imidazole.

For purification of HMG-14(1-73)/GAL4, HMG-14(1-73)/HMG-2, and GAL4AD1, the harvested bacteria were resuspended in buffer L (50 mM NaH_2PO_4 [pH 8.0], 300 mM NaCl, 7 mM β-mercaptoethanol, 0.1% Nonidet P-40, 4 μg of leupeptin per ml, 4 μg of aprotinin per ml, 1 mM phenylmethylsulfonyl fluoride [PMSF]) and lysed by sonication. Clarified supernatants were absorbed onto Ni-NTA resin (Qiagen), which was washed with buffer N100 (20 mM Tris-HCl [pH 7.5], 100 mM NaCl, 10% glycerol, 0.5 mM PMSF) plus 30 mM imidazole. The HMG-14 derivatives and His-G4AT1 were subsequently eluted with buffer N100 plus 100 mM imidazole. HMG-14(1-73)/GAL4 was further purified by chromatography through a Q-Sepharose column (Pharmacia). After washing with buffer N100, the fusion protein was eluted with buffer N200 (same as N100 except with 200 mM NaCl). Overexpression and purification of His-tagged HMG-2 were essentially as described previously (54), except that only Ni-NTA affinity column chromatography was performed.

For purification of T7 · HMG-14(1-73)/C24, the harvested bacteria were resuspended in buffer L and lysed by sonication. After clarification, the majority of this fusion protein was in the insoluble pellet, which was suspended by using a Dounce homogenizer in 6 M guanidine-HCl–25 mM Tris-HCl (pH 7.8)–25 mM NaCl–5 mM β-mercaptoethanol–20 mM imidazole, incubated for 1 h, and clarified. Solubilized T7 · HMG-14(1-73)/C24 was purified by Ni-NTA affinity column chromatography with buffers containing 8 M urea as suggested by the manufacturer. Peak fractions were pooled and dialyzed sequentially against refolding buffer (1 M urea, 25 mM Tris-HCl [pH 8.0], 1 mM dithiothreitol [DTT], 0.5 mM EDTA, and 0.5 mM PMSF) and storage buffer (see below). To control for the effect of the denaturing process on the activity of T7 · HMG-14(1-73)/C24, T7 · HMG-14 was purified by the same procedures. In this case, the bacterial pellet was directly resuspended in the 6 M guanidine-HCl solution, since this protein was soluble in buffer L after sonication and clarification.

All protein preparations were more than 90% pure and were dialyzed into storage buffer (20 Tris-HCl [pH 7.5], 50 mM NaCl, 0.5 mM PMSF, 0.5 mM EDTA, 1 mM DTT, and 10% glycerol), frozen in liquid nitrogen, and stored at −80°C. Protein concentrations were determined by the bicinchoninic acid protein assay (Pierce) with bovine serum albumin (BSA) as a reference. Compared to other colorimetric assays, the bicinchoninic acid assay is less dependent on the specific amino acid residues in each protein, permitting standardization of the HMG concentrations with BSA.

In vitro transcription. Histone H1-depleted HeLa whole-cell extract was the source of transcription factors and pol II in all in vitro transcription assays. Histone H1 was removed from HeLa whole-cell extracts by precipitation with 2.26 M ammonium sulfate, as previously suggested (18). The in vitro transcription protocol was essentially as described previously (19) except that 170 ng of recombinant TFIIF (kindly provided by Jeff Parvin, Harvard Medical School) was added to each reaction mixture. Each reaction mixture contained 200 ng of either SV40 MCs (DNA content) or SV40 viral DNA, plus 200 ng of poly[d(I-C) · d(I-C)]; in some cases, 50 ng of pFLBH DNA, which contains the adenovirus major late promoter, was also added. For transcription assays involving T7 · HMG-14(1-73)/C24, which contains two cysteine residues, and T7 · HMG-14 (as a control), 1 mM DTT was also included. Quantitative analysis of RNA transcripts was essentially as described previously (7, 19), involving hybridization of the radiolabelled SV40 late transcripts with complementary, unlabelled single-stranded recombinant DNA containing 1,488 bases of SV40 DNA sequence (from nucleotide 294 to 1782, where the major late promoter initiates at nucleotide 325), followed by cleavage with RNase T_1. This results in the trimming of long transcripts from the circular SV40 MC templates to a maximal length with a unique 3′ terminus.

Sucrose gradient sedimentation analysis. SV40 MCs were labelled with [^{3}H]thymidine for 24 h before isolation. MCs were incubated with either BSA, full-length HMG-14, or various derivatives of HMG-14 (six molecules per nucleosome) in a 50-μl mixture containing 10 mM HEPES (pH 7.5), 50 mM KCl, 3 mM $MgCl_2$, and 1 mM ATP for 20 min at 30°C. The samples were then centrifuged through 2-ml linear sucrose gradients (15 to 30%, wt/vol) containing 50 mM NaCl or, in the case of the high-salt treatment, 0.5 M NaCl in a Beckman TLS-55 rotor at 5°C for 65 min at 40,000 rpm. After centrifugation, fractions (~110 μl) were collected, and the radioactivity in aliquots was counted in a liquid scintillation counter (Beckman).

RESULTS

HMG-14 stimulates the rate of transcriptional elongation on SV40 MCs. SV40 MCs provide an exceptional system as a model for in vivo-assembled, transcriptionally competent chromatin. We have previously demonstrated the utility of SV40 MC promoters for in vitro transcription studies (7, 8, 19). Pol II initiates transcription in vitro from the late promoter on SV40 MCs at least as efficiently as it does on naked DNA (7). Only a small percentage of the SV40 MC templates or SV40 DNA templates are utilized for transcription in vitro. However, characterization of the transcription products from the SV40 MCs in previous studies provided definitive evidence that these transcripts are generated from chromatin templates: (i) as it is in vivo, efficient transcription in vitro was dependent on the appropriate chromatin structure surrounding the promoters (8); (ii) the in vitro pattern of initiation on SV40 MCs mimicked the transcripts generated in lytically infected cells and was distinct from the in vitro pattern of initiation on SV40 viral DNA (7); and (iii) although elongation through more than 10 nucleosomes was obtained, the overall rate of elongation was lower than it was on naked DNA (18a) (see Fig. 1B). Finally, HMG-14 and HMG-17, which specifically bind nucleosomes, stimulated transcription only on SV40 MCs and not on SV40 DNA (19).

Even when added after initiation of transcription, HMG-14 enhanced the production of SV40 late transcripts from SV40 MCs (19), demonstrating the ability of HMG-14 to stimulate elongation. To assess the percentage of the elongating pol II complexes on the chromatin templates that are stimulated by HMG-14, we have now optimized the transcription reactions to maximize the number of initiated pol II complexes that transcribed at least 1,457 bases (through roughly eight nucleosomes). This required addition of recombinant TFIIF, a known elongation factor, as well as poly[d(I-C) · d(I-C)], for titration of inhibitory proteins. In this as well as all subsequent experiments, a pulse-chase transcription protocol was followed, so as to monitor only the first wave of transcription through the nucleosomes. Preinitiation complexes were allowed to form on the MCs, followed by initiation of transcription by addition of low concentrations of nucleotides, including radiolabelled UTP, for a short time (pulse), which resulted in transcripts of 100 bases or shorter. In this experiment, recombinant human HMG-14 (12) was then bound to the preinitiated chromatin templates. Finally, transcriptional elongation was facilitated by incubation with high concentrations of unlabelled nucleotides (chase). The transcripts were analyzed by hybridization to single-stranded DNA encompassing the initiation region, followed by trimming of the selected transcripts by RNase T_1 to a maximal length (7, 19). This protocol therefore both selects transcripts from a particular promoter and maps initiation sites for full-length transcripts (transcripts extending at least to the extent of the hybridizing single-stranded DNA). A representative profile of the time course of the production of SV40 late transcripts (from the 100-base initial transcripts to the full-length product of 1,457 bases) is displayed in Fig. 1A. As determined by comparing the range of RNA products at any given time point, the front of transcribing polymerases moved substantially more rapidly in the presence than in the absence of HMG-14. Although BSA was added in the absence of HMG-14 in this experiment to control for protein levels in the reaction, addition of buffer alone generated the same result (data not shown). HMG-14 therefore facilitates the progression of the majority, if not all, of the transcribing pol II complexes. In addition, HMG-14 appeared to facilitate elongation at all pause sites.

As we have previously shown (19) most of the pause sites from transcription of the SV40 MCs were chromatin specific (data not shown), indicating once again that the transcripts resulted from nucleosome-containing templates rather than from naked DNA. However, analysis of any particular pause site would not necessarily reflect the entire SV40 MC population; different chromatin templates presumably generate distinct subsets of pause sites, because nucleosomes are not specifically translationally positioned within the body of the SV40 late genes (5). Therefore, HMG-14 transcriptional effects on SV40 MCs were subsequently quantitatively evaluated by measuring the rate of production of the full-length product (Fig. 1B). This type of analysis is commonly used when studying transcriptional elongation, especially when there are multiple pause sites.

To determine whether HMG-14 could facilitate elongation on DNA templates, we used a similar experimental design. In contrast to the obvious effect of HMG-14 on transcription of MCs (Fig. 1B), no effect of HMG-14 on transcription of SV40 DNA templates was detectable, even at early times (Fig. 1B) or at specific pause sites (data not shown). Of note, no matter whether DNA or chromatin templates are used in vitro, the pol II complex has equal probability in this in vitro system of being processively elongated to long RNA (of over 1,450 bases). At later time points, even the MCs in the absence of HMG-14 generate the same percentage of full-length transcripts (19). Thus, the processivity of the pol II is not being altered, but rather the rate of elongation is. These data affirm the chromatin requirement for HMG-14 transcriptional stimulation (19) and the deduction that HMG-14 does not directly influence the pol II transcription machinery but rather influences the structure of a chromatin template.

HMG-14 alleviates histone H1-mediated inhibition of transcriptional elongation. Because the two binding sites for HMG-14 and HMG-17 on each nucleosome may overlap the single, high-affinity nucleosomal binding site for histone H1 (2), we hypothesized that HMG-14 might stimulate transcription by targeting histone H1 activities. This could be tested by determining whether HMG-14 differentially affected transcription of SV40 MCs with various amounts of histone H1. In isolated, native SV40 MCs, the molar ratio of histone H1 per core nucleosome (H1/n ratio) was approximately 0.6 (Fig. 2A, lane 2). Histone H1-depleted SV40 MCs were obtained by treatment of native SV40 MCs with 0.5 M NaCl followed by purification on sucrose gradients (core-MCs) (Fig. 2A, lane 3). Although the core nucleosomes are unaffected by a high salt concentration, this treatment obviously removes not only histone H1 but also other proteins normally associated with MCs. Thus, a critical control was to reconstitute histone H1-containing MCs from the core-MCs, by the addition of limiting amounts of histone H1, dialysis to low ionic strength, and then an additional purification to remove unbound histone H1 (core/H1-MCs) (Fig. 2A, lane 4). In this experiment, an H1/n molar ratio of 0.5 was obtained, similar to that of native SV40 MCs (Fig. 2A, lane 2). All MC preparations are essentially uniform in their histone H1 composition, as demonstrated by sedimentation analyses (e.g., see Fig. 4A). Thus, a H1/n molar ratio of 0.5 indicates that on average, one of every two nucleosomes on each MC is bound by histone H1.

To ensure that exogenously added histone H1 was appropriately positioned on the nucleosomes, the MC preparations were digested with micrococcal nuclease. Histone H1 in native chromatin is known to protect an additional 20 bp of nucleosomal DNA from digestion with this nuclease (3, 43, 55). As expected, digestion of core-MCs generated DNA fragments of 146 bp (Fig. 2B, lanes 2 to 4), while digestion of the core/H1-

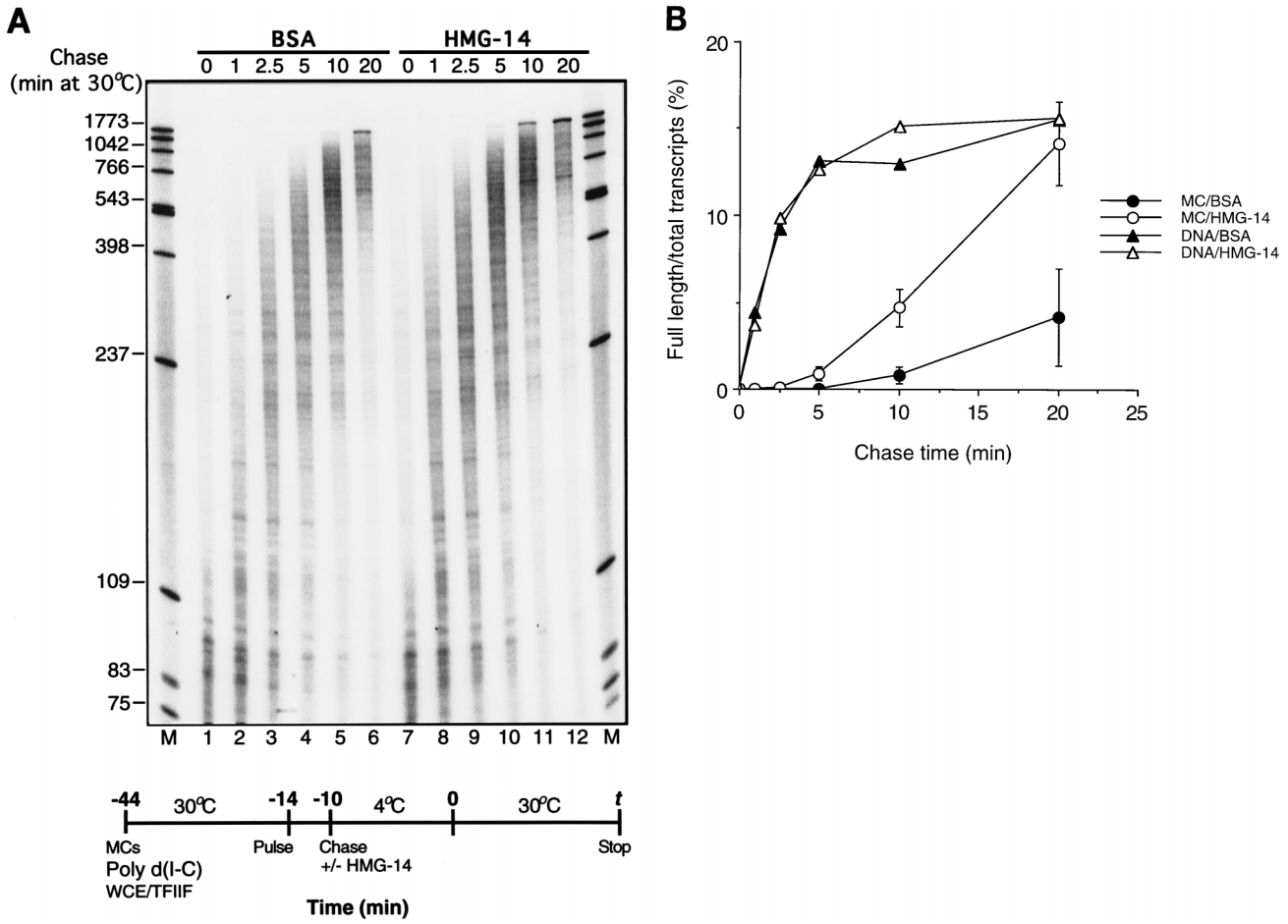

FIG. 1. HMG-14 stimulates the rate of elongation by pol II on SV40 MCs. (A) Autoradiogram of elongated transcripts from the major late promoter of SV40 MCs in the presence or absence of HMG-14. Transcription reactions were performed with histone H1-depleted HeLa whole-cell extract (WCE), as described in Materials and Methods. BSA or recombinant HMG-14 (eight molecules per nucleosome) was added following initiation of transcription. Numbers to the left of the autoradiogram indicate lengths of DNA markers (in bases). A time sequence of the protocol (with temperature conditions) is given at the bottom. The reaction mixtures were incubated for 10 min at 4°C after addition of HMG-14 in order to ensure efficient binding of HMG-14 in the absence of significant additional elongation of transcription. (B) Quantitative analysis of effects of HMG-14 on the rate of transcriptional elongation. The time-dependent generation of transcripts of 1,457 bases or longer (full length) from the major late promoter of native SV40 MCs and naked SV40 DNA was compiled either from three independent experiments (MCs) or from a representative experiment (DNA). Error bars indicate standard deviations. The percentage of these full-length transcripts relative to the total number of initiated transcripts is plotted versus time after the addition of unlabelled nucleotides. Although only 15% of transcripts were elongated to these long lengths (1,457 bases), substantially higher percentages were elongated when shorter lengths were analyzed (by hybridizing to a shorter DNA fragment). In all cases, the percentage of transcripts that are fully elongated is the same for SV40 DNA and MC templates (data not shown). In vitro transcription systems for pol II commonly result in slower and less processive elongation than under in vivo conditions (reference 30 and references therein).

MCs, which contained subsaturating levels of histone H1 per core nucleosome, resulted in accumulation of additional fragments of ~166 bp (Fig. 2B, lanes 6 to 8). In addition, upon sucrose gradient sedimentation, core/H1-MCs sedimented faster than the MCs containing no histone H1 (core-MCs) from which they were generated, indicating that addition of histone H1 compacted the MCs (Fig. 2C). Thus, by these existing criteria, exogenous histone H1 was correctly positioned on nucleosomes of the MCs.

Before testing the effects of HMG-14 on these templates, it was necessary first to characterize how histone H1 affected the transcriptional properties of the MCs. The rates of production of SV40 late transcripts from the core- and core/H1-MCs were compared, using a protocol similar to that described for Fig. 1A (except that the chase incubation immediately followed the pulse incubation). Examination of the front of transcript lengths over time indicated clearly that histone H1 retarded pol II elongation (data not shown). Quantitation of the production of the full-length SV40 late transcript with time also affirmed this inhibition, as levels of SV40 late RNA were diminished by histone H1 at short times of elongation (Fig. 3A). This was not due to inhibition of initiation of transcription from the SV40 late promoter, as the levels of transcripts after the initiation process and numbers of full-length transcripts after a long elongation incubation were unaffected by the presence of histone H1 (data not shown and Fig. 3A). The production of equivalent levels of transcripts also proves that inhibition by histone H1 was not due to precipitation of the chromatin templates or other nonspecific effects. Instead, histone H1 specifically reduced the rate of elongation by pol II by 60% under these conditions. Recently, it was reported that histone H1 also inhibited elongation through nucleosomes by T7 RNA polymerase (44).

Because HMG-14 facilitates elongation of transcription on native SV40 MCs, which contain histone H1 (Fig. 2A, lane 2), we next tested whether HMG-14 relieved inhibition of elon-

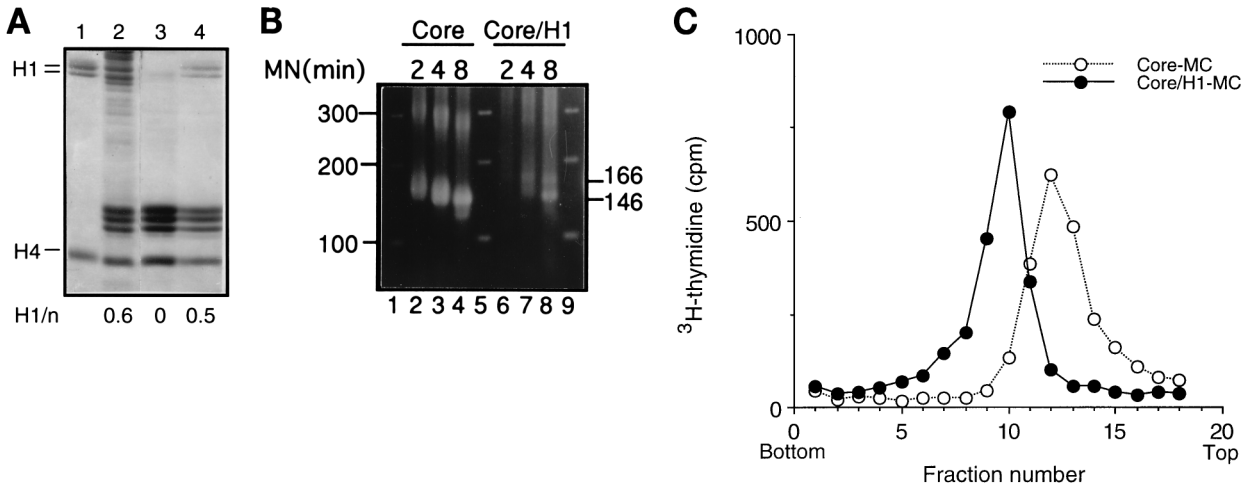

FIG. 2. Characterization of histone H1-containing and histone H1-depleted SV4 MCs. (A) Histone composition of SV40 MCs analyzed by sodium dodecyl sulfate-polyacrylamide gel (15%) electrophoresis and Coomassie blue staining. The positions of histone H1 and histone H4 are indicated. Histones H2A, H2B, and H3 migrate immediately above histone H4. H1/n ratios are indicated. Lanes: 1, purified histones H1 and H4 (1:1 molar ratio); 2, native SV40 MCs; 3, core-MCs; 4, core/H1-MCs. (B) Micrococcal nuclease (MN) digestion patterns of core-MCs and core/H1-MCs. MCs were digested for the indicated periods of time and analyzed by 4% agarose gel electrophoresis and ethidium bromide staining. Positions of the 146- and 166-bp products are indicated. Lanes 1, 5, and 9 contain DNA markers of 100, 200, and 300 bp. (C) Sedimentation profiles of core-MCs in the presence or absence of pure histone H1, as described in Materials and Methods.

gation by histone H1. The analysis was limited to effects of HMG-14 on elongation by delaying addition of HMG-14 until after transcription was initiated. Identically to its effects on native SV40 MCs, HMG-14 enhanced the rate of elongation of the majority, if not all, of the pol II on the core/H1-MCs (see Fig. 1A for protocol; data not shown). Full-length products were quantitated in multiple experiments at a time point at which the transcriptional elongation effects of HMG-14 could be analyzed readily (15 min) (Fig. 1 and 3A). Addition of HMG-14 dramatically increased the levels of full-length transcripts from histone H1-reconstituted MCs (core/H1-MCs) at this time point (Figs. 3B and C). (Transcriptional enhancement saturates upon addition in vitro of between two and six molecules of HMG-14 per nucleosome [19], consistent with saturation of the two binding sites per nucleosome. Native SV40 MCs as isolated contain minimal amount of HMG-14; the 100,000 copies of SV40 MCs per nucleus are presumably in large excess over the amounts of free HMG-14.) In sharp contrast, histone H1-depleted core-MCs were transcriptionally inert to HMG-14 (Fig. 3B and C). In fact, HMG-14 quantitatively reversed the inhibition of pol II elongation mediated by histone H1 (Fig. 3C). Because HMG-14 enhanced transcription from core/H1-MCs that were reconstituted from unresponsive core-MCs by the sole addition of pure histone H1, we conclude that the presence of histone H1 is not only necessary but also sufficient, when appropriately positioned in the context of SV40 MCs, to achieve HMG-14-mediated transcriptional stimulation. The ability of HMG-14 to counteract histone H1 was specific; addition of either HMG-1 or HMG-I/Y, both small, highly charged chromosomal proteins, did not alleviate the transcriptional inhibition by histone H1 (data not shown).

HMG-14 relieves histone H1-mediated structural compaction of SV40 MCs. Given the well-established ability of histone H1 to promote the folding and compaction of chromatin (22, 65) and the inverse correlation between chromatin compaction and efficient transcription (27, 32), we tested whether transcriptional enhancement of SV40 MCs by HMG-14 could be due to reversal of histone H1-mediated chromatin compaction. Alterations in chromatin structure were assayed by sedimentation of SV40 MCs through a sucrose gradient (3, 61), using the same buffer and cation concentrations as in the transcription reactions. A more condensed structure is reflected by a faster sedimentation through the gradient, and conversely, a more extended structure sediments more slowly (61). As expected, removal of histone H1 from native MCs by high-salt treatment led to a dramatic reduction in sedimentation rate through the gradient (Fig. 4A, top). Addition of HMG-14 to native SV40 MCs (Fig. 4A, top) or to core/H1-MCs (data not shown) also consistently reduced the sedimentation rate of the chromatin, suggesting induction of an extended chromatin conformation. The effect of HMG-14 on the sedimentation rate was smaller than that induced by the removal of histone H1 (Fig. 4A, top), as discussed further below.

To probe the link between the abilities of HMG-14 to stimulate transcription and to extend chromatin structure, we analyzed the sedimentation profile of core-MCs, which are transcriptionally unresponsive to HMG-14, in the presence or absence of exogenous HMG-14. Purified core-MCs sedimented much more slowly than native SV40 MCs (Fig. 4A, bottom), indicative of a highly extended structure. Addition of HMG-14 did not alter the sedimentation profile of the histone H1-depleted core-MCs. The correlation between structural and transcription effects was also upheld by the inability of HMG-1, transcriptionally inert on SV40 MCs, to alter the sedimentation profile of the native MCs (data not shown). Taken together, these results establish that HMG-14 affects both the higher-order structure and the transcriptional potential of histone H1-containing SV40 MCs, but not of histone H1-depleted MCs. In prior studies (61), it was shown that incorporation of HMG-14 during chromatin assembly in a *Xenopus* extract could also result in less-compact chromatin, even in the absence of histone H1 (and without apparent influence of the B4 protein). However, the binding of HMG-14 to preassembled chromatin, either SV40 MCs (this study) or bulk chromatin (26), will unfold compacted chromatin only if linker histones are present. Thus, HMG-14 and HMG-17 may display two mechanisms for reducing chromatin compaction: one apparently dependent on core histone tails (61) and the other dependent on the presence of a linker histone (this study and reference 26).

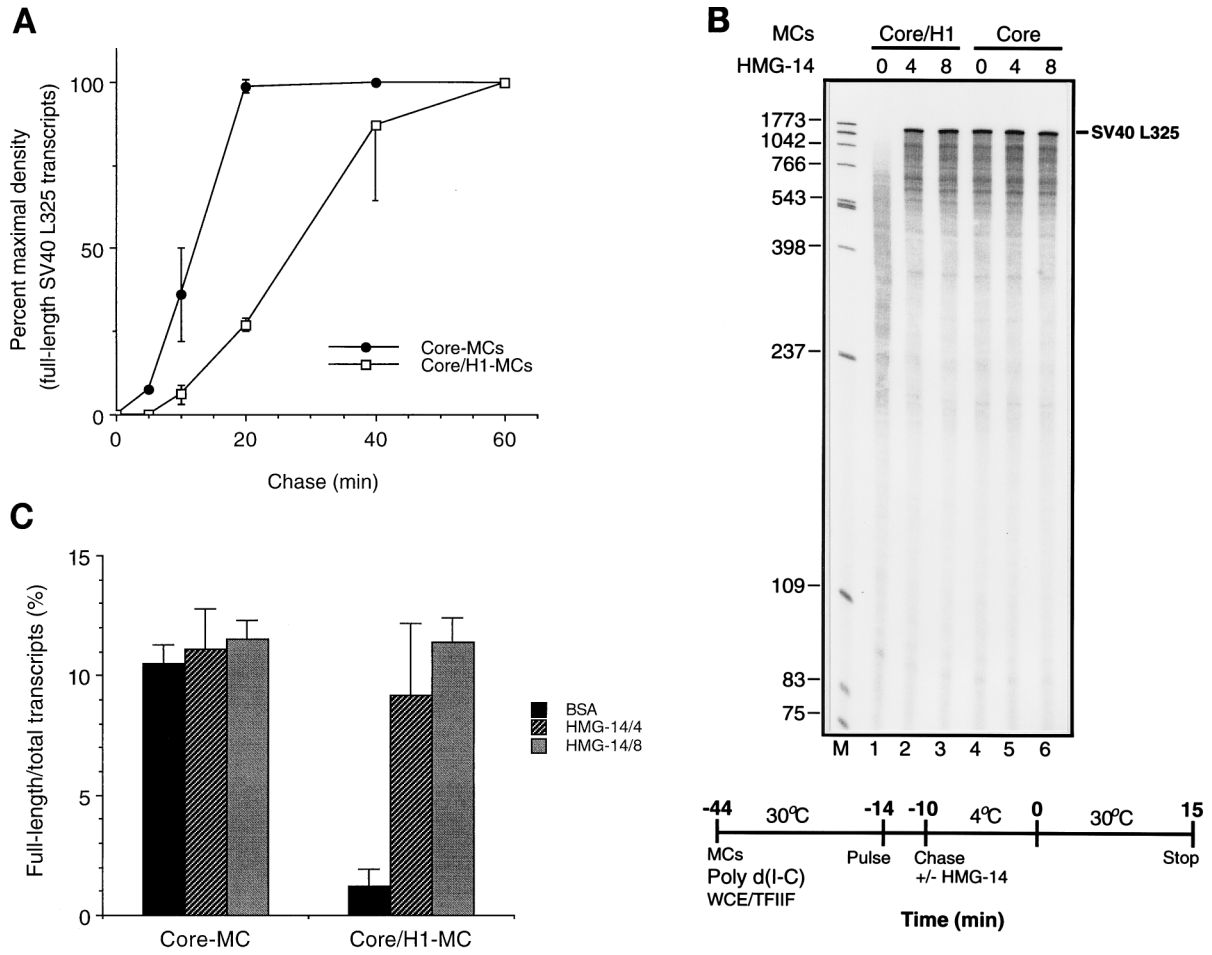

FIG. 3. HMG-14 alleviates histone H1-mediated inhibition of elongation by pol II. (A) Quantitative analysis of effects of histone H1 on the rate of transcriptional elongation. The time-dependent generation of transcripts of 1,457 bases or longer from the major late promoter of core- and core/H1-MCs (SV40 L325) were compiled from three independent experiments. The levels of SV40 MC transcripts in each reaction were normalized to the internal control transcripts from naked adenovirus major late promoter DNA (19) and are plotted versus time after the addition of unlabelled nucleotides. Levels of MC transcripts are presented as percent maximal density, that is, the level relative to the maximal plateau level obtained in that experiment. Error bars indicate standard deviations. (B) Autoradiogram of elongated transcripts from representative in vitro transcription reactions with core-MCs and core/H1-MCs. Full-length transcripts from the major late promoter of the SV40 MCs (SV40 L325) are indicated. BSA (lanes labelled 0) or the indicated amount of recombinant HMG-14 (four or eight molecules per nucleosome) was added following initiation of transcription. Numbers to the left of the autoradiogram indicate lengths of DNA markers (in bases). A time sequence of the protocol (with temperature conditions) is given at the bottom. The reaction mixtures were incubated for 10 min at 4°C after addition of HMG-14 in order to ensure efficient binding of HMG-14 in the absence of significant elongation of transcription. Transcripts were analyzed at a single time point (15 min) of elongation in the presence of unlabelled nucleotides. WCE, whole-cell extract. (C) Quantitative analysis of effects of HMG-14 on histone H1-mediated inhibition of transcriptional elongation. Bars represent the mean ratio (percentage) of full-length late SV40 transcripts to the total number of late SV40 transcripts, averaged from five independent experiments (including the experiment whose results are shown in panel B). Error bars indicate standard deviations.

To determine whether HMG-14 reduced histone H1-mediated compaction by dissociating histone H1 from the chromatin, we determined the histone and HMG protein compositions of native SV40 MCs, in the presence or absence of exogenous HMG-14, after purification by sucrose gradient sedimentation. As shown in Fig. 4B (lanes 4 to 6), the presence of exogenous HMG-14 did not alter the level of associated histone H1. The apparent increase in the intensity of the histone H1 bands was due to a sharpening of the MC peak in the gradient by HMG-14 (Fig. 4A, top) and therefore an increased concentration of chromatin. This consistent sharpening of the peak suggests that addition of HMG-14 reduces the number of conformational states available to the MCs. The retention of histone H1 upon HMG-14 addition makes the decrease in sedimentation rate even more striking, as addition of more mass alone, in the absence of conformational alterations, would result in a higher rate of sedimentation. In sharp contrast to the retention of histone H1 on slower-sedimenting MCs containing HMG-14, the slower sedimentation of salt-treated MCs resulted from complete loss of histone H1 (Fig. 4A, top, and Fig. 4B, lanes 7 to 9). These data indicate that HMG-14 must unfold chromatin templates by directly or indirectly counteracting histone H1 interactions that lead to chromatin compaction. The retention of histone H1 is consistent with the degree of decondensation by HMG-14 (assessed by sedimentation changes) being less extreme than when histone H1 is lost. In all respects, our sedimentation data mirror previous detailed structural neutron-scattering studies of the effects of HMG-14 on bulk chromatin (26), as discussed more fully below.

The acidic C-terminal region of HMG-14 is responsible for both transcriptional enhancement and chromatin decompaction. Chromosomal protein HMG-14 possesses a modular structure, consisting of a short N terminus with a net positive charge (+2; residues 1 to 12), a central domain that has a net

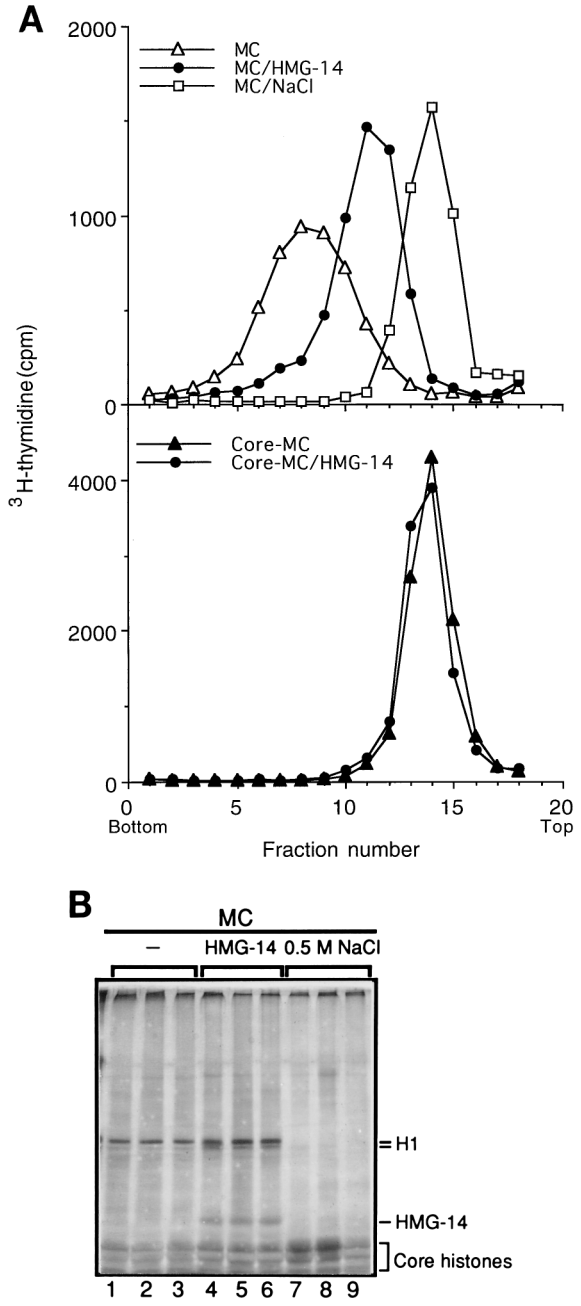

FIG. 4. HMG-14 alters the structure of histone H1-containing MCs. (A) Sedimentation profiles of native SV40 MCs (top) with no treatment or in the presence of recombinant HMG-14 (six molecules per nucleosome) or of 0.5 M NaCl and of core-MCs (bottom) in the presence or absence of recombinant HMG-14. The [^{3}H]thymidine content of the MCs in each fraction is plotted versus fraction number. Note the cosedimentation of 0.5 M NaCl-treated MCs (top) with core-MCs (bottom). (B) Histone and HMG-14 compositions of native SV40 MCs in the peak fractions of each gradient obtained from the experiment whose results are shown in panel A. —, with BSA (fractions 7 to 9); with HMG-14, fractions 10 to 12; with 0.5 M NaCl, fractions 13 to 15. Proteins were analyzed by sodium dodecyl sulfate-polyacrylamide gel (12.5%) electrophoresis and silver staining. The positions of histone H1, HMG-14, and core histones are indicated.

positive charge (+13; residues 13 to 43) and is responsible for specific binding to nucleosomes (16), and a C-terminal region with a net negative charge (−3; residues 44 to 99). Although the acidic C terminus appears to be characteristic of acidic transcription activators, neither HMG-14 nor the related HMG-17 activated pol II transcription in yeast when fused to a heterologous DNA-binding domain (36). Nonetheless, the C terminus is responsible for activation of pol III transcription when HMG-14 or HMG-17 is added during chromatin assembly in a *Xenopus* extract (62). Both to examine the molecular mechanisms whereby HMG-14 alleviates the effects of histone H1 and to investigate further the connection between the transcriptional and structural functions of HMG-14, we tested several N- and C-terminal deletion mutants of human HMG-14 (Fig. 5A) for their abilities to stimulate transcription and decompact SV40 MCs. All recombinant proteins were tagged with histidines (His_6) to permit uniform purification protocols. For more effectively assessing deficiencies in the ability of HMG-14 mutants to stimulate transcription, the sensitivity of the transcription assay was enhanced by modification of the protocol. HMG-14 was incubated with the SV40 MCs prior to initiation, and the length of time of incubation with radiolabelled nucleotides was increased (to 4 min) to permit detectable elongation. With these modifications, the presence of HMG-14 not only enhanced the rate of elongation upon addition of high concentrations of unlabelled nucleotides, as described above, but also led to elevated incorporation of radioactivity per transcript, thereby amplifying the detected consequences of HMG-14. (During a shorter pulse period of 2 min, HMG-14 did not increase the levels of incorporation of labelled nucleotides into the RNA [19].)

HMG-14(12-99), lacking the N-terminal 11 amino acids preceding the nucleosome-binding domain, remained fully competent to stimulate transcription on SV40 MCs, with an efficiency comparable to that of the His-tagged, full-length HMG-14 (Fig. 5B [compare lane 5 with lane 6] and C). However, removal of the C-terminal 53 amino acids [HMG-14(1-46)] (Fig. 5B [lanes 2 and 3] and C) or 26 amino acids [HMG-14(1-73)] (Fig. 5C; see Fig. 6B, lane 8) completely abolished transcriptional activation by HMG-14. Thus, the binding of HMG-14 to the nucleosomes is insufficient for transcriptional stimulation, which requires in addition the negatively charged C-terminal tail of HMG-14.

Correlating completely with the transcriptional results, the N-terminal deletion mutant of HMG-14 decompacted chromatin structure to a similar extent as did the His-tagged, full-length HMG-14 (Fig. 5D). In contrast, the transcriptionally inactive C-terminal deletion mutant HMG-14(1-46) did not alter the sedimentation profile of SV40 MCs. These mutational results strongly suggest that the transcriptional and structural activities of HMG-14 are functionally related. Since HMG-14 and HMG-17 neither function as acidic transcriptional activators in vivo (36) nor stimulate transcription from DNA templates in vitro (19, 46, 61), we conclude that unfolding of chromatin structure by HMG-14 is the likely basis for its stimulatory effect on transcription of chromatin templates.

Heterologous acidic regions restore transcriptional and structural activities to an inactive C-terminal deletion mutant of HMG-14. The C-terminal regions of both HMG-14 (Fig. 5B and C) and HMG-17 (19a) are directly responsible for transcriptional stimulation of SV40 MC templates. While there is only limited amino acid identity, both regions are highly acidic (11, 36). We therefore speculated that the overall acidity of the C-terminal regions might be a critical feature for the transcriptional and structural activities of HMG-14 and HMG-17. This hypothesis was tested by generating two fusion proteins, HMG-14(1-73)/GAL4 and HMG-14(1-73)/HMG-2, in which the last 26 residues of HMG-14 were replaced by the acidic activation domain I of GAL4 (residues 147 to 196 with a net charge of −9 [39]) and the highly acidic C-terminal tail of

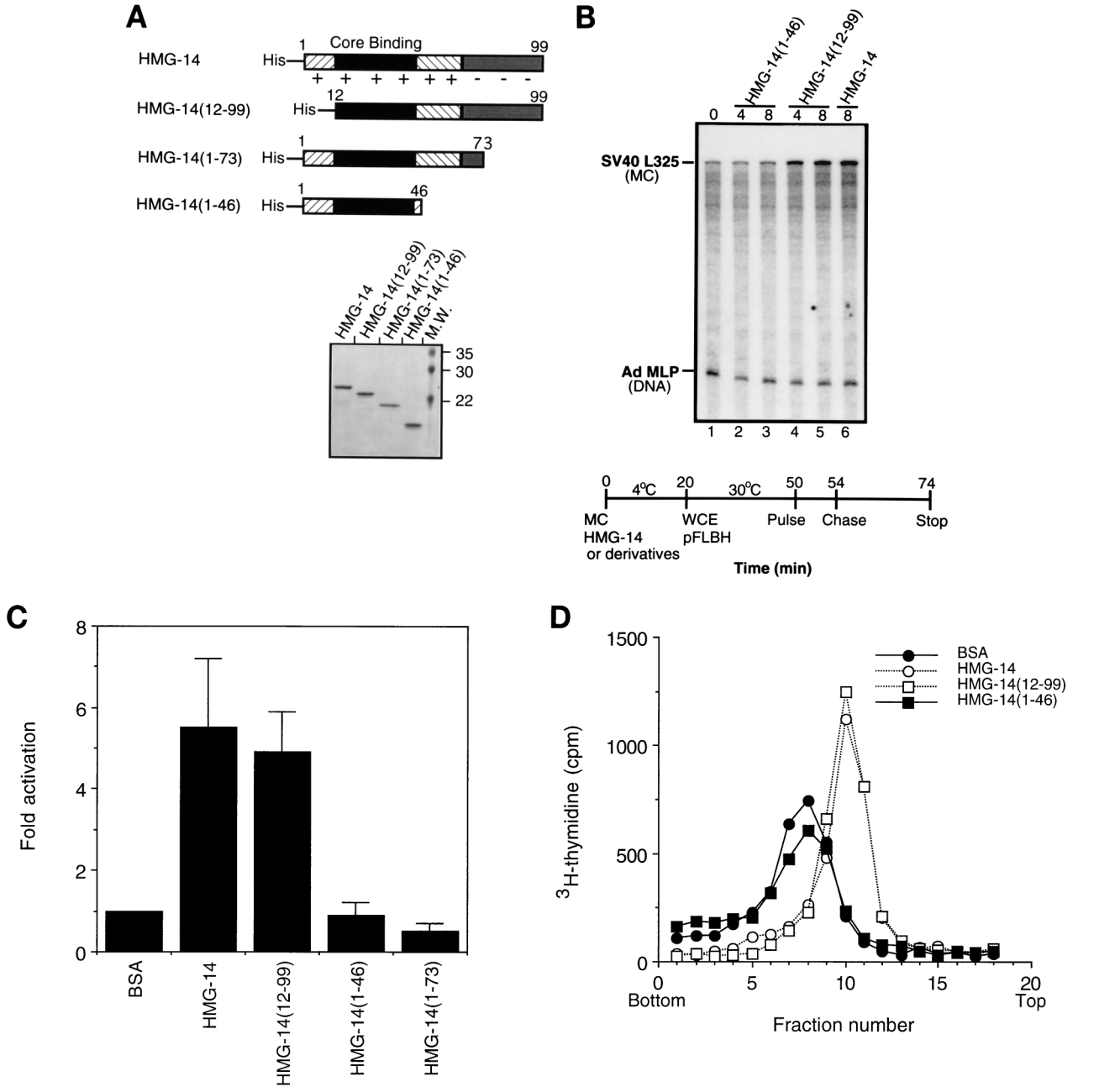

FIG. 5. The acidic C-terminal region of HMG-14 is directly responsible for both transcriptional stimulation and chromatin decondensation. (A) Top, diagram of HMG-14 and deletion mutant derivatives. Bottom, purified proteins analyzed by sodium dodecyl sulfate-polyacrylamide (15%) gel electrophoresis and staining with Coomassie blue. Numbers at the right side of the bottom panel indicate the molecular weights (M.W.) (in thousands) of marker proteins. (B) Autoradiogram of transcripts from a representative in vitro transcription experiment using His-tagged HMG-14 and its deletion mutants. Full-length transcripts from the major late promoters of SV40 MCs (SV40 L325) and of control adenovirus naked DNA (Ad MLP) are indicated (the plasmid pFLBH contains the adenovirus major late promoter [MLP]). Templates were incubated with either BSA (lanes labelled 0) or the indicated amounts of HMG-14 or its deletion mutants (four or eight molecules per nucleosome), followed by in vitro transcription. A time sequence of the protocol (with temperature conditions) is given at the bottom. WCE, whole-cell extract. (C) Quantitative analysis of effects of HMG-14 and deletion mutant derivatives on transcription of SV40 MCs. Bars represent the fold activation by these proteins, as normalized to levels of transcripts from MCs incubated with BSA. In all cases, levels of MC late transcripts were corrected for the level of transcripts from the internal control promoter (Ad MLP). The values represent averages, with indicated standard deviations, from at least five independent experiments. (D) Sedimentation profiles of native SV40 MCs in the presence of the indicated proteins (six molecules per nucleosome). The [^{3}H]thymidine content of the MCs in each fraction is plotted versus fraction number.

human HMG-2 (residues 184 to 209 with a net charge of −26 [40]), respectively (Fig. 6A). A control fusion protein, T7 · HMG-14(1-73)/C24, in which a peptide of 24 amino acids with a net charge of +3 was fused to the C terminus of the same HMG-14 deletion mutant was also generated (Fig. 6A).

Removal of the C-terminal 26 residues of HMG-14, which have a net charge of −6 (11), abolished the abilities of HMG-14 to stimulate transcription of chromatin templates (Fig. 6B [lane 8] and C) and to extend the chromatin structure (Fig. 6D). Instead, the mutant protein slightly inhibited tran-

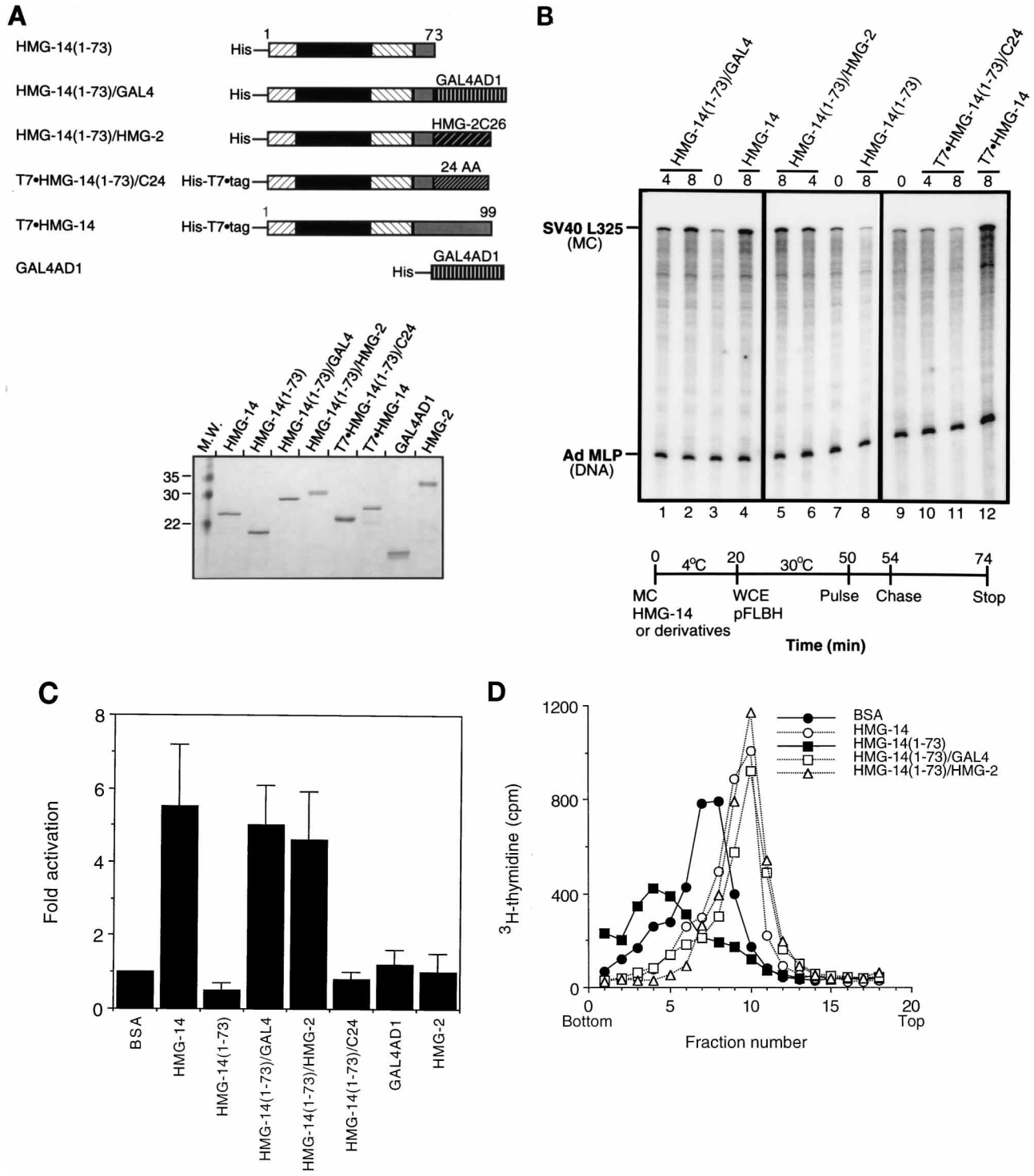

FIG. 6. The acidity of the C-terminal region is a critical feature for the transcriptional and structural activities of HMG-14. (A) Top, diagram of various fusion and control proteins. Bottom, purified proteins analyzed by sodium dodecyl sulfate-polyacrylamide (15%) gel electrophoresis and staining with Coomassie blue. Numbers at the left side of the bottom panel indicate the molecular weights (M.W.) of marker proteins in thousands. (B) Autoradiogram of transcripts from representative in vitro transcription experiments using the indicated fusion and control proteins. Full-length transcripts from the major late promoters of SV40 MCs (SV40 L325) and of control adenovirus naked DNA (Ad MLP) are indicated. Templates were incubated with either BSA (lanes labelled 0) or the indicated amounts of fusion and control proteins (four or eight molecules per nucleosome), followed by in vitro transcription. A time sequence of the protocol (with temperature conditions) is given at the bottom. WCE, whole-cell extract. T7 · HMG-14, containing the same N-terminal T7 tag (11 amino acids) as T7 · HMG-14(1-73)/C24, was functionally indistinguishable from HMG-14 in transcription assays. (C) Quantitative analysis of effects of the indicated fusion and control proteins on transcription of SV40 MCs. Bars represent the fold activation by these proteins, analyzed as described in the legend to Fig. 5C. The values represent averages and standard deviations from at least three independent experiments. (D) Sedimentation profiles of native SV40 MCs in the presence of the indicated proteins (six molecules per nucleosome). The [^{3}H]thymidine content of the MCs in each fraction is plotted versus fraction number.

scription from SV40 MCs and increased compaction of the MCs. This mutant has also been shown to compact chromatin when added during chromatin assembly (61). Because HMG-14(1-73) is so highly positively charged, it is likely to counter repulsion of negative charges on the nucleosomal DNA, as does histone H1, resulting in compaction.

Fusion of either of the two heterologous acidic domains restored transcriptional stimulation (Fig. 6B and C) and structural decompaction (Fig. 6D) activities to the HMG-14 deletion mutant. Importantly, the activities of the acidic fusion proteins depended on the nucleosome-binding domain provided by the HMG-14 deletion mutant, since neither a peptide containing the acidic activation domain I of GAL4 (GAL4AD1) nor full-length HMG-2 affected the structure (data not shown) or transcriptional potential (Fig. 6C) of SV40 MCs. These results indicate that the acidic domains must be tethered to nucleosomes to exert the demonstrated effects. In contrast, the T7 · HMG-14(1-73)/C24 fusion protein, which contains a mildly basic C-terminal region, failed to stimulate transcription (Fig. 6B [compare lanes 11 and 12] and C) and failed to decondense the MCs (data not shown). Together, these results argue that the negative charges in the C-terminal region of HMG-14 (and presumably also of HMG-17) are necessary to alleviate the transcriptional and structural effects of histone H1. This striking correlation between transcriptional and structural effects among all of the deletion and fusion proteins argues strongly that the structural changes (which are being monitored on the bulk population of MCs) also occur on the minority of templates that are being transcribed in vitro.

DISCUSSION

Our biochemical analysis of the functions of HMG-14, presented above, links the transcription-stimulatory properties of this highly conserved chromosomal protein to its ability to interfere with compaction of chromatin by histone H1. The manner in which HMG-14 affects histone H1 in vitro is consistent with the previously described properties of histone H1 on transcriptionally competent chromatin in the cell. These findings support the model that HMG-14 is a remodeling factor for the structural conversion of chromatin to an unfolded state for efficient expression of genes.

Mechanism of transcriptional activation by HMG-14. SV40 MCs have provided a facile model for mechanistic studies because their modification state and/or initial structure, reflective of transcriptionally competent cellular chromatin, permits direct responsiveness in vitro to HMG-14. On this template, histone H1 is the major repressive influence, especially as the promoter region is essentially nucleosome free. These distinctions of the in vivo-assembled MCs, compared to in vitro-reconstituted templates (discussed below), have enabled us to biochemically isolate the functions of histone H1 and determine the interplay between its activities and those of HMG-14. In particular, two important aspects regarding the mechanism of transcriptional activation by HMG-14 have been elucidated. First, we demonstrated that histone H1 is a functional target of HMG-14 in both transcriptional enhancement and chromatin decompaction, suggesting the structural-transcriptional connection. The presence of histone H1 was absolutely essential to observe either activity of HMG-14, with no effects observed on core-MCs and restoration of HMG-14 stimulation upon readdition of purified histone H1. Second, we established a direct link between transcriptional activation and extension of chromatin structure by mutational analyses. Both with deletion mutants of HMG-14 and with fusion proteins between the nucleosome-binding region of HMG-14 and exogenous peptides, these two activities of HMG-14 were inseparable. In addition, the observation that HMG-14 does not enhance transcription from naked DNA templates (Fig. 1B) (19) argues that chromatin structural alterations lead to the stimulatory effect of HMG-14 on transcription of these chromatin templates. Mechanistically, histone H1 inhibits the rate of transcriptional elongation on the SV40 MCs by enhancing chromatin-specific pausing of pol II. HMG-14 reverses this inhibition, apparently stochastically enhancing elongation through all pause sites.

The biochemical activities of HMG-14 are largely attributable to the acidic character of its C-terminal region. Indeed, the C-terminal region of HMG-14 could be replaced by heterologous acidic peptides, including an acidic activation domain of GAL4. Thus, the possibility is raised that acidic regions of other chromatin-binding proteins, such as transcription factors, may display some of the same biochemical characteristics as HMG-14, if appropriately juxtaposed to the nucleosomes. Although this is speculative, chromatin remodeling due to juxtaposition of acidic regions to specific nucleosomes in promoters might provide one component of transcriptional activation of histone H1-containing chromatin templates by GAL4-VP16 (32).

Mechanism of decondensation of the chromatin structure by the acidic domain of HMG-14. Our sedimentation analyses strongly suggested that HMG-14 partially relieved histone H1-mediated structural compaction of the SV40 chromatin templates, without displacing histone H1 from the MCs (Fig. 3). This interpretation of the sedimentation effects of HMG-14 on the transcriptionally competent SV40 MCs is completely consistent with the previous conclusions drawn from the effects of HMG-14 on the neutron-scattering properties of purified, bulk chromatin fibers either stripped of linker histones or reconstituted with linker histones (26). In that detailed structural analysis, HMG-14 was demonstrated to decrease the mass per unit length of linker histone-containing chromatin (expressed as the number of nucleosomes per 11 nm of the chromatin fiber). The HMG-14-mediated decrease in the mass per unit length of the chromatin fiber was much more subtle than the dramatic decrease in mass per unit length caused by removal of linker histones (histone H1 and H5) and the resulting decompaction of the chromatin. In addition, the mass per unit length of stripped chromatin was unaffected by HMG-14, as we found by sedimentation analyses. Because the minimal change in the radius of gyration of the HMG-14-containing chromatin fiber could not account for such the extent of the change in the mass per unit length of the chromatin fiber, Graziano and Ramakrishnan (26) concluded that HMG-14 caused an increase in the distance between neighboring nucleosomes along the chromatin fiber or, as we have interpreted our data, a decondensation of the chromatin. These structural findings mimic several characteristics of active, cellular chromatin. First, in vivo studies have shown that histone H1 remains associated with transcriptionally active genes (9, 20, 31), although in some instances histone H1 density is lower on active than on inactive genes. Thus, both histone H1 and HMG-14 or HMG-17 are likely to coexist on active chromatin. Indeed, analysis of single nucleosomes derived from in vitro reconstitution studies showed that two molecules of HMG-14 or HMG-17 and one molecule of histone H1 could cooccupy a single nucleosome (1). Furthermore, it has been demonstrated that the interaction between histone H1 and nucleosomes in active chromatin is altered (42, 67). This corresponds precisely to our finding that histone H1 can no longer fully compact chromatin in the presence of HMG-14 or HMG-17. Although other mechanisms may also modify histone H1-chromatin interactions on

the transcriptionally competent chromatin (both in vitro and in vivo), our data indicate that HMG-14 and HMG-17 provide one mechanism to mediate this transition.

Compaction of chromatin is substantially driven by electrostatic forces, including neutralization of the negative charges of the linker DNA by the basic tails of histone H1 (14). Interactions between histone H1 and adjacent histone H1 molecules and/or adjacent nucleosomes may also contribute to the formation of higher-order structure (48, 67, 73). Taking these considerations into account, we propose three models for how HMG-14 might counteract histone H1. First, the acidity of HMG-14 may reduce the shielding of negative charges along the linker DNA by the basic tails of histone H1, therefore restoring the electrostatic repulsion that tends to extend chromatin structure. In support of this electrostatic model, the negative charge of the C-terminal region or heterologous domains, when tethered to a nucleosome via the nucleosome-binding domain of HMG-14, is sufficient to decondense chromatin (Fig. 4A and 6D).

A second possibility is that HMG-14 may interact directly with histone H1, altering its charge interactions or changing its conformation such that histone H1 interactions with adjacent histone H1 molecules or with nucleosomes are modified. The feasibility of an interaction between histone H1 and HMG-14 or HMG-17 is suggested by the overlap of binding sites of HMG-14 and HMG-17 with that of histone H1 near the dyad axis of the nucleosome (2). Third, HMG-14 may interact directly with only the core nucleosome, altering its configuration in subtle ways (50, 64) either to modify its interactions with histone H1 or to inhibit its ability to stack onto adjacent nucleosomes. These three models are clearly not mutually exclusive.

Differential access of chromatin to HMG-14 and HMG-17 proteins and biological implications. A role for the chromosomal proteins HMG-14 and HMG-17 in transcriptional potentiation of chromatin templates in vitro has been demonstrated in two types of model chromatin systems, each of which has different potential biological implications. The amount of HMG-14 or HMG-17 per nucleus is sufficient to bind to only a subset of the nucleosomes, constituting at most 10% of the nucleosomes (25). A critical issue relating to the biological function of HMG-14 and HMG-17 is how these proteins can be targeted to specific regions of chromatin for transcriptional induction. One model for access of this family of proteins to specific genes suggests that chromatin assembly following DNA replication may allow preferential incorporation into early-replicated DNA (13), which is enriched in transcriptionally active genes (24, 28, 66). This mechanism would predetermine the association of HMG-14 and HMG-17 proteins with transcriptionally competent chromatin. Such a model is supported by experiments involving reconstitution of chromatin templates in vitro, in which HMG-14 or HMG-17 accesses nucleosomes only during DNA replication and/or de novo chromatin assembly. Either *Xenopus* egg extract (17, 61) or *Drosophila* embryo extract (46) assembly systems normally produce a transcriptionally repressed chromatin structure (32, 61). Incorporation of HMG-17 or HMG-14 alters this highly repressed state, stimulating transcription initiation by pol II (46) and transcription turnover by pol III (17, 61).

However, transcriptional and structural activities of HMG-14 and HMG-17 on preassembled, transcriptionally competent SV40 MCs (reference 19 and this study) indicate that HMG-14 and HMG-17 can also directly bind and modify in vivo-assembled SV40 minichromosomes in a chromatin assembly-independent manner. Such a mode of access for HMG-14, in particular, is consistent with a potential role in the rapid regulation of gene expression in response to a variety of extracellular signals. As opposed to HMG-17, HMG-14 is specifically and rapidly phosphorylated in response to both mitogenic stimuli and thyroid-stimulating hormone (6, 56) and is rapidly acetylated in response to estrogen (10, 47).

In vitro nucleosome binding assays indicate that each nucleosome has two high-affinity binding sites for either HMG-14 or HMG-17 (1, 2, 41, 53). Consistent with these structural data, two or more molecules of HMG-14 or HMG-17 per nucleosome are required for maximal transcriptional activation on chromatin templates in both in vitro-reconstituted and in vivo-assembled chromatin systems (17, 19, 46, 61). In addition, the transcriptional phenotypes of HMG-14 and HMG-17 deletion mutants are extraordinarily similar in both systems (this study and reference 62). Even a single-amino-acid substitution mutant of HMG-14, in which the glutamic acid at residue 76 is altered to glutamine, is reduced in transcriptional activation potential to similar extents with either *Xenopus* extract-reconstituted chromatin or SV40 MCs (19a, 62). This convergence strongly suggests that the structural bases for transcriptional stimulation are identical. Thus, whereas access of HMG-14 and HMG-17 to chromatin can be differentially controlled, once these proteins are bound to nucleosomes, chromatin is similarly unfolded, increasing the accessibility of the template to transcription processes.

In summary, our data suggest a novel molecular model for configuring chromatin structure in the absence of DNA replication and nucleosome assembly to increase the transcriptional potential of histone H1-containing chromatin templates. We propose that association of HMG-14 with nucleosomes in specific regions of chromatin, mediated either by specific transcriptional activators or by histone acetylation, would counteract histone H1 activity so that the local chromatin structure would become more extended. This would facilitate elongation by pol II and would ensure productive transcription.

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