

# Modular Structure of Chromosomal Proteins HMG-14 and HMG-17: Definition of a Transcriptional Enhancement Domain Distinct from the Nucleosomal Binding Domain

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**Chromosomal proteins HMG-14 and HMG-17 are the only known nuclear proteins which specifically bind to the nucleosome core particle and are implicated in the generation and/or maintenance of structural features specific to active chromatin. The two proteins facilitate polymerase II and III transcription from in vitro- and in vivo-assembled circular chromatin templates. Here we used deletion mutants and specific peptides to identify the transcriptional enhancement domain and delineate the nucleosomal binding domain of the HMG-14 and -17 proteins. Deletion of the 22 C-terminal amino acids of HMG-17 or 26 C-terminal amino acids of HMG-14 reduces significantly the ability of the proteins to enhance transcription from chromatin templates. In contrast, N-terminal truncation mutants had the same transcriptional enhancement activity as the full-length proteins. We conclude that the negatively charged C-terminal region of the proteins is required for transcriptional enhancement. Chromatin transcription enhancement assays, which involve binding competition between the full-length proteins and peptides derived from their nucleosomal binding regions, indicate that the minimal nucleosomal binding domain of human HMG-17 is 24 amino acids long and spans residues 17 to 40. The results suggest that HMG-14 and -17 proteins have a modular structure and contain distinct functional domains.**

Considerable experimental evidence suggests that the transcriptional activity of genes is related to their chromatin structure and that active chromatin is enriched in nonhistone proteins and less condensed than transcriptionally inactive chromatin (31, 41, 42). Chromosomal proteins HMG-14 and HMG-17 may play a role in the generation of structural features which are unique to transcriptionally active genes (12). In immunofractionation experiments these proteins are preferentially found in chromatin regions enriched in transcribed genes (19, 20, 34) and in acetylated histones (29). Antibodies to HMG-17 inhibit transcription when injected into nuclei (21). Both HMG-14 and HMG-17 enhance transcription from a chromatin template assembled in a *Xenopus* egg extract, but not from a naked DNA template, suggesting that the proteins act only in the context of chromatin (17, 39, 40). Similar results were obtained by using the simian virus 40 minichromosome as a template, in which case HMG-14 enhanced transcription by stimulating elongation but not initiation (18). In templates assembled in *Xenopus* egg extracts, incorporation of HMG-14 and -17 into nascent nucleosomes induces an unfolded chromatin structure (40).

HMG-14 and -17 are the only nuclear proteins known to bind specifically to the nucleosome core particle (2, 3, 30, 36). Thus, their ability to increase the transcriptional potential of chromatin templates is most probably related to their ability to bind to nucleosomes. Analysis of the primary structure of HMG-14 and -17 indicates that the proteins are built of distinct structural domains (12). We have suggested that these structural domains may represent functional modules within the protein (16, 25). Indeed, one of these domains is the primary site of interaction with nucleosomal DNA (1, 8, 14, 33). Furthermore, a peptide with an amino acid sequence corresponding to this domain retains many of the nucleosome binding

properties characteristic of the intact protein (16). However, in contrast to the full-length protein, this peptide does not enhance transcription from a chromatin template assembled in a *Xenopus* egg extract (40). These findings strengthen the possibility that HMG-14 and -17 are modular proteins, with different domains performing different functions. In an attempt to identify the segment(s) of the proteins necessary for transcriptional enhancement, we constructed N- and C-terminally truncated proteins and tested their ability to enhance transcription of chromatin templates assembled in *Xenopus* egg extracts. Here we show that the negatively charged C-terminal domain of HMG-14 and -17 is required for transcriptional enhancement. In addition, we delineate the nucleosome binding domain of these proteins by means of a transcription enhancement assay that involves binding competition between the full-length proteins and peptides derived from their nucleosomal binding regions.

## MATERIALS AND METHODS

**Construction of the expression vectors.** Vectors for the expression of truncated as well as full-length HMG-14 and -17 proteins were prepared by PCR from the cDNAs coding for the proteins (26, 27). The PCR products were cloned into the *NdeI*-*BamHI* sites of the T7 polymerase expression vector pVEXII (a gift from A. Shankar, National Institutes of Health). The sequences of the constructs were verified by dideoxy sequencing.

**Expression of the proteins.** *Escherichia coli* BL21(DE3) was transformed with the expression constructs, and cultures were grown in Luria-Bertani medium containing 100 µg of ampicillin per ml to an optical density at 600 nm of 0.6. Expression was induced by adding 0.4 mM isopropylthiogalactoside. The incubation was continued for 2 to 3 h. The HMG-14 and -17 proteins were extracted from bacterial pellets with 5% perchloric acid and further purified as described before (10, 33). All the proteins used in this study were over 90% pure, as determined by polyacrylamide gel electrophoresis (PAGE) and by high-performance liquid chromatographic analysis.

**Gel mobility shift assay.** The interaction of HMG-14 and -17 proteins with nucleosome core particles was studied by gel mobility assays essentially as described before (33). The concentration of core particles was 100 nM in a solution containing 2× Tris-borate-EDTA, 5 mM dithiothreitol, and 0.1 mg of acetylated bovine serum albumin (BSA) per ml. Recombinant proteins were added from stock solutions made in 20 mM Tris-HCl (pH 7.5)–5 mM dithiothreitol–0.1 mg

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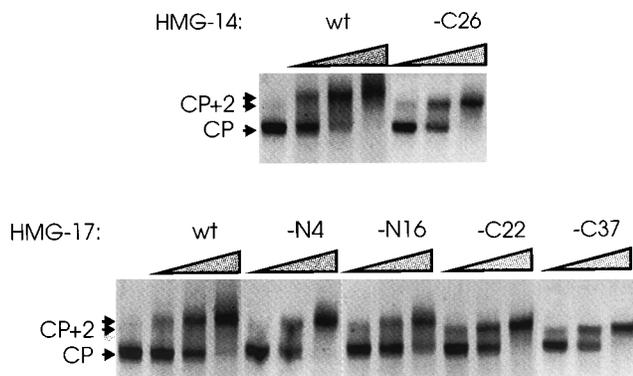


FIG. 2. Binding of full-length and truncated HMG-14 and -17 proteins to nucleosome cores. Core particles were incubated under cooperative binding conditions with various concentrations of HMG proteins and deletion mutants. The molar ratios of protein to core particles were approximately 1, 2, or 4. The resulting complexes (core particles [CP] and core particles plus two HMG molecules [CP+2]) were resolved on native 5% acrylamide gels and stained with ethidium bromide. All proteins bind with similar affinities to the cores. The arrows indicate the mobilities of the core particle-HMG complexes.

that neither the C-terminal regions nor the four conserved N-terminal amino acid residues of the proteins are necessary for binding to nucleosome cores.

**Analysis of the transcription enhancement capability of the truncated HMG-14 and -17 proteins.** We have demonstrated that in a *Xenopus* egg extract chromatin assembly system incorporation of HMG-14 and -17 into the nascent nucleosomes increases the transcription potential of the resulting chromatin template (17, 40). We used this experimental system to determine which of the conserved protein domains is involved in the transcription enhancement function of this protein family. Minichromosomes were assembled in the egg extract in the presence of various concentrations of truncated or full-length protein and transcribed in the presence of [<sup>32</sup>P]CTP (Fig. 3). For each experimental set the relative amount of transcript was normalized to that synthesized in the absence of added HMG protein. The data in Fig. 3A indicate that the amount of 5S rRNA transcript obtained from chromatin assembled in the presence of HMG-17 was 6.1-fold greater than that obtained from chromatin assembled in the absence of HMG-17 (compare lanes 1 and 3 in Fig. 3A). The ability of HMG-17 to enhance transcription was significantly reduced by the deletion of the last 22 (Fig. 3A) or 37 (data not shown) C-terminal amino acids. This result suggests that the negatively charged C-terminal segment of HMG-17 is required for transcriptional enhancement. Likewise, the transcription enhancement activ-

TABLE 1. Dissociation constants for binding of HMG-14 and -17 proteins and mutants to nucleosome cores under cooperative binding conditions

Protein	Dissociation constant
HMG-14	$1.05 \times 10^{-7}$
HMG-17	$0.44 \times 10^{-7}$
HMG-14(76Q)	$0.7 \times 10^{-7}$
(-C26)HMG-14	$0.7 \times 10^{-7}$
(-C22)HMG-17	$0.51 \times 10^{-7}$
(-C37)HMG-17	$0.55 \times 10^{-7}$
(-N4)HMG-17	$0.55 \times 10^{-7}$
(-N16)HMG-17	$1.0 \times 10^{-7}$
Peptide 2	$0.98 \times 10^{-7}$

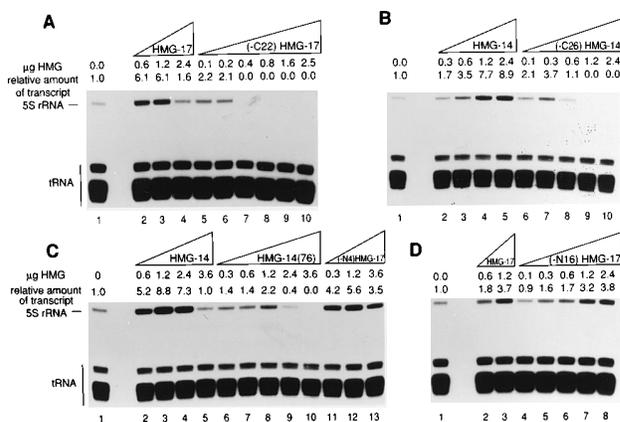


FIG. 3. Effect of truncated HMG-14 and -17 proteins on the transcriptional potential of chromatin. Minichromosomes were assembled in the egg extract for 2 to 2.5 h at 22°C in the presence of the various HMG proteins as indicated in the figure (1.2 μg of full-length protein corresponds to a molar ratio of HMG to nucleosomes of about 50; however, since there are two HMG molecules per nucleosome, the ratio of HMG to HMG-binding sites is 25). [<sup>32</sup>P]CTP and unlabelled ribonucleotides were then added, and after 30 min the radiolabelled transcripts were purified, electrophoresed on sequencing gels, and autoradiographed. Note that both the C-terminally truncated proteins (panel A, lanes 5 to 10, and panel B, lanes 6 to 10) and the HMG-14(76) mutant protein (panel C, lanes 6 to 10) failed to significantly increase transcription of the 5S rRNA gene. In contrast, the N-terminally truncated proteins fully retained their enhancement capability (panel C, lanes 11 to 13, and panel D, lanes 4 to 8). In each panel the relative amount of transcript is normalized to that synthesized in the absence of HMG protein. The radioactivity in the tRNA region, resulting from nucleotide exchange, serves as a control for experimental variations.

ity of the C-terminally truncated HMG-14 protein was significantly lower than that of the intact HMG-14 protein (Fig. 3B). However, this truncation retained some of the enhancement activity (Fig. 3B), suggesting that in HMG-14 the enhancement domain may be somewhat longer. Replacement of a glutamic acid at position 76 of HMG-14 with glutamine (33) resulted in an almost complete loss of the ability to activate transcription (Fig. 3C), suggesting that this region is the only one necessary for transcription enhancement. This mutation is located in a highly conserved region and may disrupt the secondary structure of the C-terminal domain.

Consistent with our previous observations (17, 40) we found that higher concentrations of HMG-14 and -17 inhibit transcription of both chromatin (Fig. 3A, lane 4, and 3C, lane 5) and naked DNA (data not shown). Interestingly, the C-terminally truncated proteins inhibit transcription at considerably lower concentrations. These proteins do not affect the solubility of the templates; however, they inhibit the rate of chromatin assembly. These truncated mutants also inhibit transcription from naked DNA (data not shown). This inhibition may involve nonspecific interactions between the HMG-14 and -17 proteins and either the transcriptional machinery or the transcribed template. Deletion of the negatively charged segment and the resulting increase in net positive charge of the HMG proteins may strengthen this effect.

Next, we tested the activity of the N-terminally truncated HMG-17 proteins in the transcription enhancement assay. Both the (-N4)HMG-17 deletion mutant and the (-N16)HMG-17 deletion mutant stimulate transcription of the chromatin template to an extent similar to that of stimulation by the full-length protein (Fig. 3C and D). However, because the affinity constant of the (-N16)HMG-17 mutant for binding to nucleosome cores is twofold lower than that of the intact protein (Table 1), the amount of this mutant necessary to fully

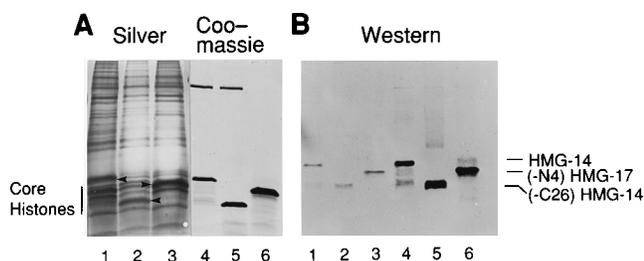


FIG. 4. Binding of full-length and truncated HMG-14 and -17 proteins to in vitro-assembled minichromosomes. Minichromosomes were assembled in the egg extract in the presence of the HMG proteins indicated in the figure. The minichromosomes were purified by centrifugation through a sucrose gradient, and their protein contents were analyzed by SDS-PAGE. Each sample was split, and half of the gel was first stained with Coomassie blue (A); the other half was analyzed by immunoblotting with an antibody against the conserved DNA binding domain of HMG-17 (B). For panel A, after the gel was stained with Coomassie blue it was restained with silver to better visualize the HMG-14 proteins present in the assembled minichromosomes (arrowheads in lanes 1 to 3). Lanes 1, 2, and 3 contain the minichromosomes assembled in the presence of HMG-14, (-C26)HMG-14, and (-N4)HMG-17, respectively. Lanes 4 to 6 contain the purified HMG proteins. The high-molecular-weight protein in lanes 4 and 5 of panel A is BSA.

stimulate transcription was twofold greater (compare lanes 3 and 8 in Fig. 3D). We conclude, therefore, that the protein region which is N terminal to the nucleosomal binding domain, including the invariant first four N-terminal amino acid residues, is not involved in the transcription enhancement function of HMG-14 and -17.

Although the results shown in Fig. 2 indicate that the C-terminal truncation mutants can interact with isolated nucleosome cores, it is still possible that the C termini are necessary for incorporation into chromatin during nucleosome assembly. Therefore, we checked whether the truncated mutant proteins are indeed incorporated into chromatin during the in vitro assembly reaction in the *Xenopus* egg extract. We selected the (-N4)HMG-17 and (-C26)HMG-14 proteins and, as a control, the full-length HMG-14 protein for these experiments. Minichromosomes were assembled in the egg extract in the presence of these HMG proteins and purified on sucrose gradients, and their protein contents were analyzed by SDS-PAGE and Western blotting (Fig. 4). Both truncated proteins (lanes 2 and 3) were incorporated into the minichromosomes as efficiently as the full-length protein (lanes 1). We conclude therefore that the proteins are assembled into chromatin. These results also support the conclusions reached from the mobility shift assays (Fig. 2) and indicate that the nucleosome binding function of the truncated proteins is intact.

**Delineation of the nucleosomal binding domain.** A 30-amino-acid peptide (named peptide 2) comprising the DNA binding region of HMG-17 (amino acids 17 to 46) interacts specifically with nucleosome core particles (16) but has no stimulatory effect on transcription of chromatin (40). In an assembly mixture containing both peptide 2 and the full-length protein, the HMG-17-mediated transcriptional stimulation was inhibited (Fig. 5A, lanes 1 to 4), most probably because the peptide competed with the wild-type protein for binding to the nucleosomes. Peptide 3 (from the C-terminal domain of HMG-17; Fig. 5C) and peptide C (a control peptide, with a sequence different from that of peptide 2 but an amino acid composition identical to that of peptide 2), which do not bind to nucleosomes, had no effect on transcription (lanes 6, 13, and 14). As expected from the similarities in their amino acid sequences (Fig. 5C), peptide 2 also competed with HMG-14 (lane 12). Conversely, a peptide corresponding to the binding

region of HMG-14, named peptide 4, competed with both HMG-14 (lanes 7 to 11) and HMG-17 (lane 5), corroborating earlier results suggesting that both proteins have the same nucleosome binding sites (3). Further evidence for the specificity of this reaction is obtained by analysis of peptide 7 (Fig. 5C), in which a highly conserved alanine at position 21 was mutated to proline. We have previously reported that this point mutation reduces by sevenfold the binding constant of the full-length proteins with isolated nucleosome cores (33). In the context of the peptide, this mutation significantly reduces the ability of the peptide to inhibit the HMG-17-mediated transcriptional enhancement of chromatin templates (Fig. 5B, lanes 2 to 4).

Chromosomal proteins HMG-14 and -17 enhance transcription only from chromatin templates and do not affect the transcription of naked DNA. Likewise, the peptides inhibited only the transcription from chromatin templates containing HMG-14 and -17 and did not affect the transcription of a control gene (*Sat I*) which was added as naked DNA together with the [<sup>32</sup>P]CTP used to measure transcription (Fig. 5). We conclude therefore that the transcriptional inhibition of the various peptides was due to competition with the wild-type HMG-14 and -17 proteins for binding to chromatin templates and not to nonspecific effects on the transcriptional process.

<sup>1</sup>H nuclear magnetic resonance studies with the entire

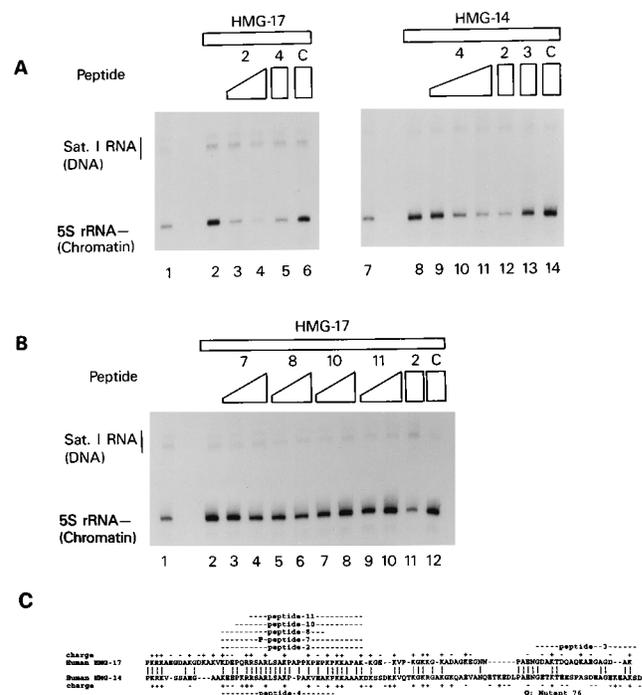


FIG. 5. Delineation of the nucleosome binding domain of HMG-14 and -17. Minichromosomes were assembled from single-stranded DNA in the egg extract in the presence of HMG-14 and -17 and the peptides shown in panel C, and then transcription was carried out in the presence of [<sup>32</sup>P]CTP. As a control for transcription from naked DNA, a double-stranded plasmid carrying the *Sat I* gene from *X. laevis* was added together with the labelled CTP. The amount of HMG-14 and -17 added was 1.2 μg, and the amounts of peptides were as follows for panel A: peptide 2, 1.2, 5, and 5 μg in lanes 3, 4, and 12, respectively; peptide 4, 5, 0.6, 1.2, and 5 μg in lanes 5, 9, 10, and 11, respectively; and peptides C and 3, 5 μg. For panel B, 5 and 10 μg of peptides 7, 8, 10, and 11 and 5 μg of peptides 2 and C were used. Peptide C has the same composition as peptide 2 but a randomized sequence. Mutant 76 denotes the Glu-to-Gln exchange at position 76 of the HMG-14 sequence (33). This mutant protein was used for Fig. 3C. Note that peptides 2 and 4 inhibit the enhancement of transcription from chromatin but do not affect the transcription of the unchromatinized *Sat I* template.

HMG-17 molecule (14) and mobility shift assays with peptides (16) suggested that the main nucleosomal binding site is confined to the positively charged, central region of the protein. The ability of peptides derived from the nucleosomal binding regions of the protein to inhibit the stimulatory effect of HMG-14 and -17 on transcription of chromatin templates provides a simple way to delineate more precisely the nucleosomal binding domain of this protein family. In this way we tested several truncated forms of peptide 2 (Fig. 5C). N-terminally truncated mutants of peptide 2 (peptide 10 and peptide 11, lacking three and six residues, respectively) failed to compete with HMG-17 (Fig. 5B, lanes 7 to 10), suggesting that the minimal binding domain starts with amino acid residue 17, 18, or 19 of human HMG-17. Peptide 8, a C-terminally truncated peptide 2 lacking six residues, competed partially with the protein (lanes 5 and 6). However, peptide 4, which is derived from the nucleosome binding region of HMG-14 and extends two amino acids further towards the C terminus than peptide 8, competed fully with HMG-14 and HMG-17 (Fig. 5A). Assuming identical lengths for the binding domains of HMG-14 and HMG-17, the C-terminal end of the binding domain of human HMG-17 is at position 40, which corresponds to residue number 35 of human HMG-14.

## DISCUSSION

The main finding reported in this article is that the negatively charged C-terminal domain of HMG-14 and -17 is necessary to stimulate transcription from in vitro-assembled chromatin templates. These results provide further evidence that chromosomal proteins HMG-14 and -17 have a modular structure and that their evolutionarily conserved structural domains correspond to distinct functional motifs.

**Functional domains in HMG-14 and -17 proteins.** The two properties most commonly associated with HMG-14 and -17 proteins are specific binding to nucleosome cores and some type of involvement in the generation of transcriptionally active regions in chromatin (12). Analysis of the primary structure of this protein family revealed that the amino acid residues previously identified by nuclear magnetic resonance studies as the main sites of interaction with nucleosome cores and DNA (1, 14) are clustered into an evolutionarily conserved domain and that the C-terminal region of the molecule contains several evolutionarily conserved motifs and has a net negative charge (12, 25). These features suggest that the proteins have a modular structure and that the structural domains may be associated with various functions of the proteins. This suggestion is supported by the data presented here (summarized in Fig. 6) and by previous studies in which mutant proteins (33) and various peptides (16) were used to study the structure-function relation of this protein family. As diagrammed in Fig. 6, row 1, wild-type HMG-14 or HMG-17 proteins bind to nucleosomes and can enhance transcription. Replacement of a glutamic acid by glutamine at position 76 (row 2) did not affect the binding of the protein to nucleosomes (33) but significantly reduced the protein's transcription enhancement ability (Fig. 3). Deletion mutants lacking the first four amino acids (Fig. 6, row 3) or even the entire segment preceding the conserved nucleosomal binding domain (row 4) bound to nucleosome cores and enhanced transcription. On the other hand, the C-terminal deletion mutants (rows 5 and 6) bound to nucleosomes (Fig. 2) and were assembled into chromatin (Fig. 4) but failed to enhance transcription. Peptide 2 (Fig. 6, row 7), corresponding to the entire nucleosomal binding region of HMG-17 (Fig. 1A), binds to nucleosomes (16), is incorporated into chromatin (data not shown), fails to enhance

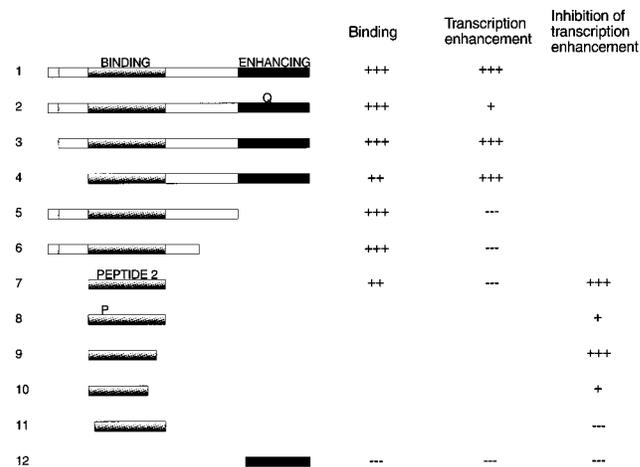


FIG. 6. Functional domains in HMG-14 and -17 proteins. The schematic diagram summarizes the studies suggesting that the nucleosomal binding function and the transcription enhancement function of the HMG-14 and -17 proteins reside in different structural domains. The binding of the proteins was measured by mobility shift assays. The transcription enhancement function was determined by measuring the transcription potential of chromatin, assembled in *Xenopus* egg extracts, either in the presence or in the absence of HMG protein. The inhibition of transcription enhancement was detected by assessing the ability of a peptide to inhibit the transcription enhancement of the full-length protein. Row 1, intact protein (the shaded region at the N terminus corresponds to the first evolutionarily conserved domain [Fig. 1]); row 2, HMG-14 protein bearing a Glu-76-to-Gln point mutation; rows 3 and 4 and 5 and 6, the two N-terminal and two C-terminal HMG-17 truncation mutants shown in Fig. 1, respectively; row 7, a 30-amino-acid peptide (peptide 2) corresponding to the nucleosomal binding domain of HMG-17; row 8, peptide 2 in which the alanine at position 9 was mutated to proline. Rows 9 to 12 correspond, respectively, to peptides 4, 8, 10, and 3 in Fig. 5C. +++, ++, +, or +, strong, medium, or weak activity, respectively; ---, no activity.

transcription (40), and, as expected, also inhibits the ability of the wild-type protein to enhance transcription, suggesting that it competes with the wild-type protein for binding to nucleosome cores. This result supports our previous suggestion that the nucleosomal binding domain can act as an independent functional domain and that it binds properly to nucleosome cores (16). Indeed, DNase I digestion (16) and hydroxyl radical footprinting studies (unpublished data) indicate that the placement of this peptide in nucleosome cores is very similar to that of the wild-type protein. Furthermore, studies with mutated proteins indicated that point mutations in this domain significantly impair the ability of the wild-type protein to bind to nucleosome cores. Indeed, deletion of the three N-terminal amino acids (Fig. 6, row 11) from peptide 2 abolishes its ability to compete with the wild-type protein in the transcription enhancement assay. Even a single amino acid substitution, from alanine to proline (Fig. 6, row 8, and Fig. 5C), significantly reduces the ability of the peptide to compete with the entire protein. On the other hand, deletion of the six C-terminal amino acids (Fig. 6, row 9) had no effect, and deletion of the last eight amino acids (Fig. 6, row 10, and Fig. 5C) had only a limited effect on the inhibition of transcription enhancement. These studies map the nucleosomal binding domain and indicate that the N terminus of this domain is the lysine residue at position 17 in HMG-17 (residue 13 in human HMG-14). Deletion of the 22 C-terminal residues of HMG-17 reduced significantly the ability of the protein to enhance transcription. In fact, even a single amino acid change from glutamic acid to glutamine at position 76 of HMG-14 drastically reduced the ability of the protein to enhance transcription. This residue is located in a conserved domain (domain 4 [Fig. 1A]), suggesting

that this region is necessary for the transcription enhancement activity.

In contrast to the positively charged central domain, the acidic domain (Fig. 6, row 12) does not bind to DNA (1, 12), and a peptide from this region fails to bind to the nucleosome core particle (16). However, through interactions with the core histones, this region may still contribute to the binding of HMG-17 to the core particle (14), perhaps through nonspecific electrostatic interactions. Our finding that the removal of 1 negative charge of 12 in HMG-14 significantly reduces the transcription enhancement effect argues against this possibility and suggests that the interaction between the HMG-14 and -17 enhancement domain and the histones has stringent structural requirements. Other studies have also supported the assumption that although HMG-14 and -17 are unstructured random coils in solution (1, 8, 13), they assume a more ordered structure when bound to the nucleosome (33).

Cross-linking experiments indicate that the HMG-14 and -17 proteins interact with the core histones; however, these interactions have not been mapped in detail (9, 15). Recent evidence that HMG-14 and -17 may facilitate transcription of chromatin by inducing a more unfolded chromatin conformation (40) led us to suggest that HMG-14 and -17 may interact with the N-terminal tails of the core histones, which are believed to be involved in the folding of the nucleosome chain into higher-order chromatin conformations (4, 22). The results presented here suggest that the negatively charged C-terminal domain of HMG-14 and -17 may be primarily responsible for this interaction.

**A negatively charged transcription-enhancing domain.** The modular structure of HMG-14 and -17 is reminiscent of that of acidic transcription activators (23) in that both have a distinct binding domain and a negatively charged transcriptional enhancement domain. The acidic regions of these transcriptional activators seem to play a role in recruiting basal transcription factors and promoting the assembly of preinitiation complexes (7, 35, 38). In addition, these activators may alleviate nucleosome-mediated repression of DNA replication (28) and activate transcription by disrupting the nucleosomes in promoter regions of genes (37). In contrast to these acidic transcription factors, HMG-14 and -17 bind to nucleosomes in chromatin without any specificity for the DNA sequence and do not seem to be part of the transcription complex. These proteins should be considered structural components which enhance the transcriptional potential of a chromatin template by unfolding the higher-order chromatin structure. An unfolded chromatin structure may be utilized more efficiently by the various components of the transcription apparatus. Since the proteins preferentially bind to nucleosome cores and affect transcription only in the context of chromatin and not in that of naked DNA, it is likely that the change in the chromatin structure is mediated by their interaction with the histones in nucleosomes. Hydroxyl radical footprinting analysis suggests that these proteins may affect the binding of histone H1 to chromatin (3). However, in *Xenopus* egg extracts the binding of HMG-14 and -17 does not reduce the amount of the H1 homolog B4 bound to minichromosomes (40). Therefore, we have suggested that HMG-14 and -17 may affect the higher-order chromatin structure by interacting with the positively charged core histone tails. On the basis of the results presented here, it seems likely that these interactions involve the acidic terminal regions of the HMG-14 and -17 molecules. We note, however, that in cellular chromatin, interactions of HMG-14 and -17 proteins with histone H1 and with the core histone tails are not necessarily mutually exclusive. Both of these interactions could syn-

ergistically act to reduce the compactness of the chromatin fiber and facilitate access to the DNA.

What is the functional significance of a multidomain structure in HMG-14 and -17 proteins? HMG-14 and -17 bind specifically to nucleosome core particles, and therefore they can be considered an integral part of the chromatin fiber. Multiple specific interactions between the protein and nucleic acid components in chromatin facilitate complex processes such as replication, DNA repair, recombination, and transcription. So far most of the data suggest that HMG-14 and -17 proteins are not directly involved in any of these processes and are not an integral part of the transcription complex. However, these proteins may still act as "architectural" elements and facilitate transcription in the context of chromatin, perhaps by negating the repressive effect of histones and inducing structural changes that ease the binding of other factors to their targets. Their modular structure may reflect a requirement for interacting with both the DNA and the histones in nucleosomes and may facilitate multiple cooperative interactions which increase the overall flexibility of a multicomponent structure such as the transcribing chromatin fiber.

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