MOLECULAR AND CELLULAR BIOLOGY, Sept. 1991, p. 4483–4489
0270-7306/91/094483-07$02.00/0

Vol. 11, No. 9

Assessment of the Transcriptional Activation Potential of the HMG Chromosomal Proteins

DAVID LANDSMAN[1]* AND MICHAEL BUSTIN[2]

National Center for Biotechnology Information, National Library of Medicine, Building 38A, Room 8N-807, Bethesda, Maryland 20894,[1] and Laboratory of Molecular Carcinogenesis, National Cancer Institute, Bethesda, Maryland 20892[2]

Received 8 January 1990/Accepted 19 June 1991

Chromosomal proteins HMG-14, HMG-17, and HMG-1 are among the most abundant, ubiquitous, and evolutionarily conserved nonhistone proteins. Analysis of their structure reveals features which are similar to those of certain transcription factors. The distribution of charged amino acid residues along the polypeptide chains is asymmetric: positive charges are clustered toward the N-terminal region, while negative charges are clustered toward the C-terminal region. The residues in the C-terminal region have the potential to form α helices with negatively charged surfaces. The abilities of HMG-14, -17, and -1 to function as transcriptional activators were studied in *Saccharomyces cerevisiae* cells expressing LexA-HMG fusion proteins (human HMG-14 and -17 and rat HMG-1) which bind to reporter molecules containing the β-galactosidase gene downstream from a *lexA* operator. Fusion constructs expressing deletion mutants of HMG-14, -17, and -1 were also tested. Analysis of binding to the *lexA* operator with in vitro-synthesized fusion proteins shows that there are more sites for HMG-14, -17, and -1 binding than for LexA binding and that only the fusion constructs which contain the C-terminal, acidic domains of HMG-17 bind the *lexA* operator specifically. None of the LexA-HMG fusion protein constructs elevate the level of β-galactosidase activity in transfected yeast cells. Thus, although HMG-14, -17, and -1 are structurally similar to acidic transcriptional activators, these chromosomal proteins do not function as activators in this test system.

The chromatin structure of actively transcribed genes is different from that of bulk chromatin (for reviews, see references 2, 13, 16, and 37). It has been suggested that chromosomal proteins HMG-14 and -17 may confer unique properties to transcriptionally active chromatin regions (48–50). This hypothesis is supported by results obtained from DNase I digestion studies (50), reconstitution experiments with isolated nucleosomes (33, 39, 40), experiments which indicate that microinjection of anti-HMG-17 antibodies into somatic cells inhibits transcription (12), immunofluorescence studies which indicate that antibodies to HMG-14 preferentially stain active regions of polytene chromosomes (53), immunofractionation experiments indicating that chromatin regions enriched in HMG-17 are enriched in transcriptionally active genes (9) and in acetylated histones (31), and immunoprecipitation experiments indicating that HMG-17 is associated with actively transcribed genes (7, 8). However, the cellular functions of these proteins remain obscure, since several laboratories have failed to reveal a correlation between their presence and the transcriptional state of a gene (for reviews, see references 13 and 21). Similarly, chromosomal proteins HMG-1 and HMG-2 may be associated with actively transcribed regions of chromatin. Microinjection of antibodies to HMG-1 and -2 into living oocyte nuclei of *Pleurodeles walthii* caused retraction of transcription loops (24). In vitro transcription assays indicate that HMG-1 and HMG-2 can increase the rate of binding of transcription factors to their DNA recognition sequences, presumably by mediating the formation of active transcription initiation complexes (42, 45, 47).

Certain structural features of the HMG proteins are evolutionarily conserved (2, 6, 23, 27, 28, 43, 46, 52), suggesting that they may be important for the cellular functions of these proteins. Here we demonstrate that the structures of the HMG-14 and HMG-17 proteins are reminiscent of certain transcription factors such as GAL4, GCN4, and VP16 (for reviews, see references 10, 34, 36, 41, and 44). The charged amino acids are asymmetrically distributed along the polypeptide chains, the positive residues are clustered toward the N-terminal regions, and the C-terminal regions are negatively charged. Here we test the potential of HMG-14, HMG-17, and HMG-1 to function as transcriptional activators.

MATERIALS AND METHODS

Strains. *Saccharomyces cerevisiae* KY803 (*trp1-Δ1 ura3-52 leu2-P1 gcn4-Δ1*), constructed by Joan Sellers and obtained from K. Struhl, was used for transformation with the various fusion protein constructs by the lithium acetate method (22). The same plasmids were used for transformation of *Escherichia coli* DH5α cells by the rubidium chloride method (32). These plasmid preparations were used for sequencing and for transformation of yeast cells.

Culture media and growth conditions. Bacterial cells were grown at 37°C overnight in YT medium. Yeast cells were grown at 30°C in SD medium supplemented with adenine, histidine, lysine, and tryptophan or on plates containing the same medium. Yeast cells grown for the purpose of extracting total proteins were grown in YPD medium.

Preparation of LexA-HMG fusion protein constructs. A shuttle vector containing selectable markers for both *E. coli* and *S. cerevisiae* was obtained from K. Struhl (18, 19). The vector, YCp88-lexA-gcn4Δ19, contains a constitutive promoter from the yeast *DED1* gene which initiates the synthesis of the *lexA-GCN4* mRNA and fusion protein. The *GCN4* coding region was removed from this plasmid by restriction

* Corresponding author.

with *Sal*I and *Eco*RI and, after fill-in of the ends with DNA polymerase Klenow fragment, HMG coding region inserts were ligated into the blunt ends of the vector. All HMG coding regions were prepared by polymerase chain reaction since there are no convenient restriction sites. The upstream amplimers were identical to regions starting with the first base of the codon of the first amino acid of the required HMG coding region. Downstream amplimers were complementary to the regions starting 12, 11, and 16 bases downstream of the stop codons of the cDNAs encoding human HMG-17, human HMG-14, and rat HMG-1, respectively (27, 28, 35). Each of the latter amplimers contained a 5′-extending *Eco*RI site, thereby creating a unique site convenient for identification of clones containing appropriate inserts. The sequence of the complete HMG insert, including the junction of the two coding regions, was verified by double-stranded sequencing in both directions, using oligonucleotides identical and complementary to different regions of the *lexA*, HMG-14, HMG-17, and HMG-1 reading frames (data not shown).

β-Galactosidase assays. To assay the transcription activation potential of the various LexA-HMG fusion proteins, yeast cells (strain KY803) were cotransfected with both a fusion protein construct and the reporter construct containing the *lexA* operator upstream from the β-galactosidase gene, and transformants were selected for on Leu$^-$ Ura$^-$ Ade$^+$ His$^+$ Lys$^+$ Trp$^+$ SD plates. Plasmids YCp88-lexA-gcn4Δ19, pY176, pY177, pY142, pY143, pY146, pY11, pY12, pY15, and pX, a negative control expressing essentially the LexA part of the fusion proteins, were successfully transfected. Overnight cultures of the yeast cells were diluted 1/1,000 and grown to an optical density at 600 nm of about 1.0. Three 1-ml aliquots from two separate clones containing each of the fusion protein constructs were assayed for β-galactosidase activity (17).

Northern (RNA) analysis and preparation of total yeast RNA. RNA was prepared from yeast cells containing fusion constructs by the sodium dodecyl sulfate (SDS)/phenol/glass bead method exactly as described elsewhere (30). Northern analysis was performed as previously described (27).

Western immunoblot analysis and preparation of total yeast protein. Total protein was isolated from yeast cells by boiling in SDS. Cells were grown overnight and were then pelleted and washed in distilled water. Cells were then resuspended in 300 μl of distilled H_2O containing 1 mM phenylmethylsulfonyl fluoride, and 150 μl of 3× sample application buffer for SDS-polyacrylamide gels was added. The samples were boiled for 3 min, cooled, centrifuged, and then stored at 4°C. Aliquots (25 μl) were applied to 15% SDS-gels (25). Proteins were transferred to nitrocellulose filters by electroblotting according to the instructions of the manufacturer. These filters were blocked in 3% bovine serum albumin (BSA) in phosphate-buffered saline. Antibodies against the first 87 amino acids of LexA (a gift from J. Little) were used at a dilution of 1/1,000 in 5% BSA–Tris-buffered saline (TBS; 10 mM Tris-HCl [pH 7.4], 150 mM NaCl)–1% Triton X-100–1% sodium deoxycholate and were incubated with the filter for 2 h at room temperature. Filters were washed twice in TBS with 0.05% Tween 20 and once in TBS. Phosphatase-conjugated goat anti-rabbit antibody (1/1,000 dilution) was used to identify positive interactions, using a Bio-Rad visualizing kit.

In vitro transcription and translation of fusion constructs. The plasmids containing LexA fusion protein sequences contain an SP6 promoter upstream of the fusion protein-coding region (19). $CsCl_2$-purified plasmids were linearized with *Eco*RI, and mRNA was synthesized with SP6 polymerase according to the instructions of the manufacturer (Promega Corp.). The RNA was digested with DNase I, applied to a Select-D(RF) column (5′→3′, Inc.), extensively extracted with phenol/chloroform, and ethanol precipitated from ammonium acetate. The purified RNA was translated in vitro by using [^{3}H]leucine exactly as described by the manufacturer (Promega). No capping was necessary for efficient translation, as judged by SDS-polyacrylamide gel electrophoresis and fluorography of the protein products (25). Each translation reaction mix contained [^{3}H]leucine in addition to the 19 other nonradioactive amino acids. A 10-μl sample of each in vitro translation reaction mix was applied to an SDS-polyacrylamide gel. After electrophoresis, the gel was soaked in Enlightning (New England Nuclear, DuPont) and then exposed to X-ray film at −80°C.

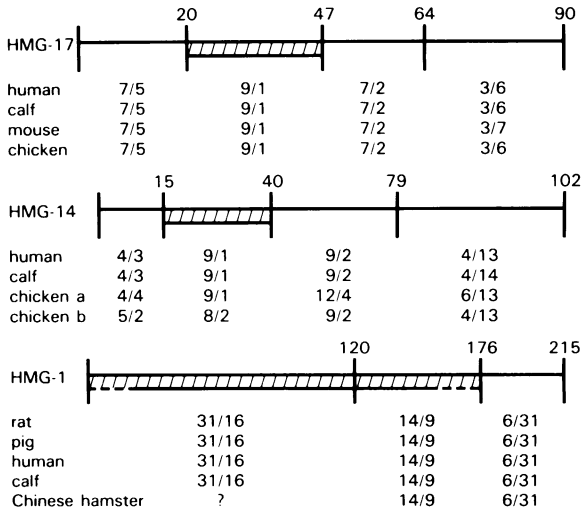

FIG. 1. Charge distribution in HMG proteins. The primary sequences of HMG-17, HMG-14, and HMG-1 are depicted as single lines, with numbers above the lines indicating amino acid residues at the boundaries of specific regions. The number of basic/acidic amino acids in each region is shown. The species from which the protein has been analyzed is shown on the left. Hatched boxes identify the DNA-binding domains of the HMGs. For HMG-1, there is uncertainty about the beginning and end of the DNA-binding domain. Regions of different charge ratios have been identified for comparison with the LexA-HMG fusion protein deletion mutation constructs (see Fig. 3).

Gel mobility shift assays of LexA fusion proteins. DNA-binding assays were performed as described by Fried and Crothers (14) except that 1.5% Ficoll was added to the incubation mix. DNA-protein interactions were performed at 37°C for 30 min. An oligonucleotide probe corresponding to the *lexA* operator (11) was synthesized and was labeled with [^{32}P]CTP by extension of a shorter oligonucleotide, complementary to the 3′ end of the operator oligonucleotide, using Klenow fragment DNA polymerase.

RESULTS

Structural features of human HMG-14, human HMG-17, and rat HMG-1. Analysis of the primary structure of the known HMG proteins reveals a remarkable conservation in the distribution of charged residues along the polypeptide chain (Fig. 1). For comparative analysis, each of the proteins was divided into regions differing in net charge. From Fig. 1

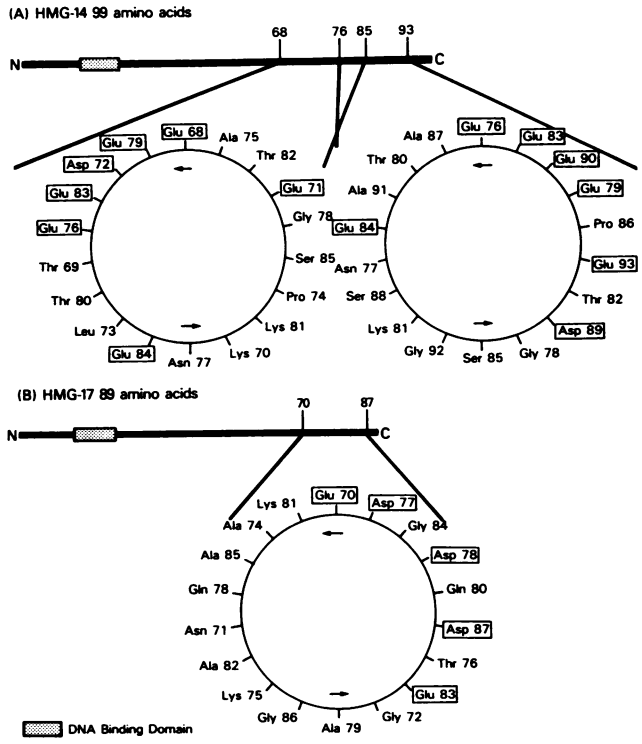

FIG. 2. Helical wheel projections of peptides from HMG proteins. Shown is an α-helical representation of HMG-14 for residues 68 to 85 and 76 to 93 (A) and HMG-17 for residues 70 to 87 (B). The acidic amino acids, aspartic acid and glutamic acid, are boxed. The filled boxes are the DNA-binding domains. Note that the negatively charged domains are clustered on one face of the predicted α helices.

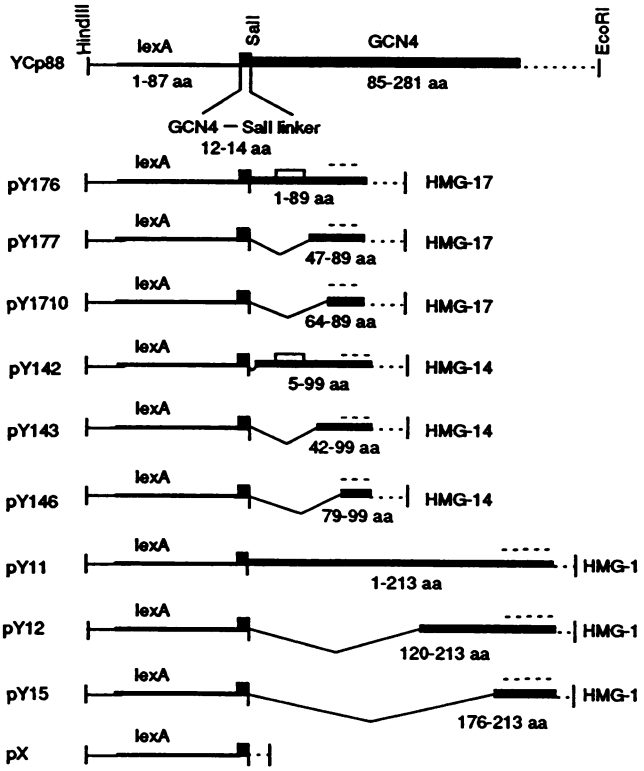

FIG. 3. LexA-HMG fusion protein constructs. Thick solid bars represent *HMG* and *GCN4* coding regions; thin solid bars represent *lexA* coding regions; solid boxes depict the GCN4-*SalI* linkers. The 5′ untranslated regions are thin lines, and the 3′ untranslated regions thin dotted lines. Hatched boxes depict the DNA-binding domains of HMG-14 and HMG-17. Deletions in the *HMG* coding regions are depicted as thin bent lines. YCp88, pY176, pY177, pY1710, pY142, pY143, pY146, pY11, pY12, pY15, and pX are plasmids referred to in the text. Vertical lines below *Hin*dIII and *Eco*RI are the corresponding restriction sites. ---, acidic regions on HMG proteins.

it is apparent that the segment encompassing the first 20 amino acids of HMG-17 contains 12 charged amino acids with a 7/5 basic/acidic ratio. Similarly, for HMG-14, the segment encompassing the first 15 amino acids contains 7 to 8 charged residues with a net positive charge. The DNA-binding domains of HMG-17 and HMG-14 are, respectively, 25 and 27 amino acids long, and both proteins have a basic/acidic amino acid ratio of 9/1. The third segment of HMG-17 and HMG-14 is also highly positively charged. In contrast, the C-terminal 26 amino acids of HMG-17 and the corresponding 23 amino acids of HMG-14 are negatively charged. Similarly, in HMG-1, the N-terminal half of the molecule has a net positive charge of 15, the following 56 amino acids have a net positive charge of 7, and the C-terminal 40 amino acids have a net negative charge of 25. This region contains a stretch of 25 contiguous, acidic amino acids which probably do not have an ordered secondary structure. In contrast, analyses of the HMG-14 and HMG-17 protein sequences by both the Chou-Fasman (5) and Garnier-Robson (15) secondary structure prediction methods suggest stretches of α-helical structure punctuated by β-turn regions for both HMG-14 and HMG-17. Both methods predict that the negatively charged, C-terminal regions are in an α-helical conformation. Projection of these α-helical regions as helical wheels reveals regions in which the acidic residues are positioned on a single face of the helix. Examples of such regions are presented in Fig. 2. In HMG-14, between residues 68 and 85, there is a cluster of 5 negatively charged residues on one face of the α helix. Similar clusters can be observed between residues 76 and 93 of HMG-14 and between residues 70 and 87 of HMG-17. These features of the HMG proteins are reminiscent of those of transcription factors such as GCN4 and GAL4 (18, 19, 30, 38), which also have an asymmetric distribution of charge and have clusters of acidic residues. The main difference between HMG-14 and HMG-17 and transcription factors is that while the latter have the distribution of these acidic residues compatible with the formation of amphipathic α helices, the acidic regions of HMG-14 and HMG-17 will not form amphipathic helices. Because of these similarities and the previous results implicating HMG proteins in aspects of actively transcribed chromatin, we have tested the abilities of these proteins to function as transcription activators in a suitable assay system.

Construction of vectors expressing LexA-HMG fusion protein. Figure 3 is a diagram of the constructs encoding the LexA-HMG fusion proteins. All of the constructs contained at the 5′ end a short untranslated region, followed by the coding region of the first 87 amino acids of the LexA protein. The junction between this coding region and other coding sequences was created with a GCN4-*SalI* linker which contained amino acids 12 to 14 of the *GCN4* coding region ending with a *SalI* site (18, 19). LexA-HMG constructs pY176, pY142, and pY11 contained the complete coding

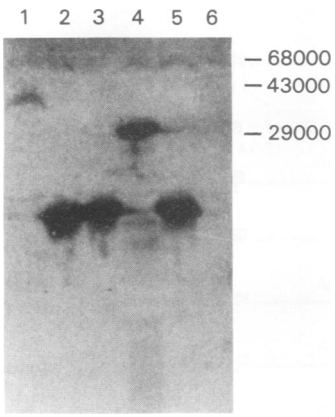

FIG. 4. Western blot analysis of fusion protein expression in yeast cells. Yeast cells containing either plasmids YCp88 (lane 1), pY176 (lane 2), pY142 (lane 3), pY11 (lane 4), and pY141 (which is identical to plasmid pY142; lane 5), each with the reporter plasmid, YEp21-Sc3423, or only the reporter plasmid (lane 6) were grown overnight. Total protein was isolated. Equal aliquots of total protein were applied to two 15% polyacrylamide gels. One gel was stained with Coomassie blue (not shown) to compare the total proteins applied to each lane, and the other gel was used for Western analysis and was probed with antibodies to the LexA portion of the fusion proteins.

regions of HMG-17, HMG-14, and HMG-1, respectively. In pY142, the first four amino acids of HMG-14 were deleted since they are identical to those in HMG-17 (i.e., PKRK). The second series of LexA-HMG constructs was identical to the first series except that the N-terminal regions containing the DNA-binding domains (1, 3, 4) of each of the proteins were deleted. These plasmids, named pY177, pY143, and pY12, code, respectively, for HMG-17 amino acid residues 47 to 89, for HMG-14 residues 42 to 99, and for HMG-1 residues 120 to 213, in addition to the N-terminal LexA domain. The lack of the HMG DNA-binding domains would minimize the possibility that the fusion proteins could interact with DNA or chromatin via these domains. The third series of LexA-HMG constructs, pY1710, pY146, and pY15, contained only the regions coding for the HMG C-terminal peptides which are rich in aspartic acid and glutamic acid (i.e., residues 79 to 99 for HMG-14, residues 64 to 89 for HMG-17, and residues 176 to 213 for HMG-1 [Fig. 1]). A negative control, pX, encodes a fusion protein in which the first 87 amino acids of LexA are fused to a 13-amino-acid peptide, KAGTIPTETIPNN. A positive control, YCp88-lexA-gcn4Δ19, encodes a LexA-GCN4 fusion protein containing amino acids 85 to 281 of GCN4 (18).

Authenticity of LexA-HMG fusion protein synthesis. Nucleotide sequencing revealed that all of the LexA-HMG constructs were accurate ligations (data not shown). To verify that the fusion products are accurately expressed, the fusion protein products from yeast strains containing plasmids YCp88-lexA-gcn4Δ19, pY176, pY142 (two clones), and pY11 were examined (Fig. 4). All lanes in the gel contained approximately equivalent quantities of protein (data not shown). Western analysis with an antibody against residues 1 to 87 of LexA shows that fusion proteins of expected molecular weights are synthesized. The calculated approximate molecular weights of the fusion proteins are 45,000, 21,100, 20,250, and 28,000 for LexA–GCN4, LexA–HMG-14, LexA–HMG-17, and LexA–HMG-1, respectively. We conclude that the constructs expressed the correct LexA-HMG fusion products. Although the total protein contents of the various samples seem to be very similar (not shown), there is a quantitative difference in fusion protein products, as seen in the Western analysis. There seems to be significantly less LexA-GCN4 fusion product than any of the LexA-HMG fusion products (Fig. 4). The difference in expressed fusion product could be due to differences in mRNA stability, protein stability, or accessibility of the antigenic determinant.

Binding of LexA-HMG fusion protein products to the *lexA* operator. Next we tested the ability of LexA-HMG fusion proteins to bind to the *lexA* operator site. ^{3}H-labeled proteins were synthesized in a cell-free system by in vitro translation of SP6 polymerase-synthesized mRNA from plasmids YCp88, pY176, pY1710, and pY11. Figure 5A shows a fluorogram of the products. Only single products were synthesized in each reaction, and each of these products was of approximately the expected molecular weight. These clones were chosen as representatives of the numerous fusion protein constructs for the following reasons. YCp88 is the positive control both for *lexA* operator binding and for its ability to activate transcription in this system (19). pY176 and pY1710 are very similar to the equivalent HMG-14 constructs pY142 and pY146 in both sequence and amino acid composition (26). In fact, the DNA-binding domains of these two proteins are identical. In addition, the modes of binding of these two proteins to DNA and nucleosomes are indistinguishable (reviewed in reference 2). Therefore, the results obtained with pY176 and pY1710 (lex-HMG-17 and lexA-HMG-17/C in Fig. 5B) would be expected to be similar to those for pY142 and pY146, respectively. pY11 is a representative of one of the HMG-1 fusion constructs.

The supernatants of the in vitro translation reactions were used to test the ability of the LexA-HMG fusion protein to bind a *lexA* operator (Fig. 5B and C). Samples of 0, 1, and 5 μl of translation reaction mixture were incubated with the ^{32}P-labeled operator DNA for 30 min at 37°C (20) (Fig. 5B). In a separate experiment (Fig. 5C), 1 μl of 1/1,000, 1/100, and 1/10 dilutions of each sample were incubated in a similar fashion. The final concentration of fusion proteins in these binding assays ranges from approximately 2 pM to 2 nM, and the approximate final concentration of DNA is 30 nM. Samples were then loaded directly onto the gel. After electrophoresis, gels were dried and autoradiographed at −80°C. In the lanes marked no lexA in Fig. 5B there were no protein products, and only nonspecific interactions, due to nonspecific binding by components present in the in vitro translation mix, are observed near the top of all other lanes in the gel. In the lanes marked lexA-GCN4 and lexA-HMG-17/C, a protein-DNA interaction band is observed, which is indicative of specific binding of the fusion protein to the *lexA* operator. The binding of LexA-GCN4 to the *lexA* operator has been shown to be specific (19). In the lanes marked lexA-HMG-17 and lexA-HMG-1, there are several bands. The relative mobilities of these bands and their intensities compared with those of the specific bands obtained with LexA-GCN4 and LexA-HMG-17/C suggest that these represent nonspecific interactions resulting from the binding of the fusion proteins through their HMG region rather than through their LexA portion. The failure of pY176 and pY11 to produce distinct bands suggests that the fusion products did not bind to the *lexA* operator plasmid in a specific manner. This may be due to the ability of the LexA-HMG fusion proteins to bind the DNA through the HMG portion of

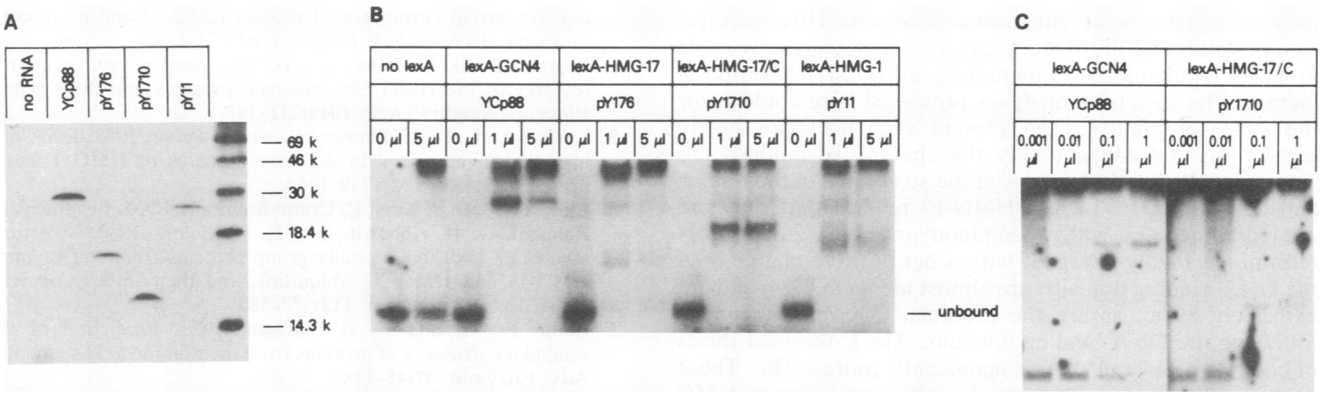

FIG. 5. Gel mobility shift assays of LexA-HMG fusion proteins. (A) Fluorograph of an SDS-polyacrylamide gel containing ^{3}H-labeled LexA fusion proteins translated from the RNA synthesized from the plasmids indicated above the lanes. A 10-µl aliquot of each translation system was analyzed. The lane marked no RNA is identical translation mix with no added mRNA. The lanes marked YCp88, pY176, pY1710, and pY11 refer to the linearized plasmid source for the RNA transcription (see Fig. 3). (B) Autoradiograph of the gel mobility shift assay. Aliquots of 0, 1, and 5 µl of the translation mix, as indicated, were incubated with ^{32}P-labeled *lexA* operator and applied to a 5% polyacrylamide gel containing a low-ionic-strength buffer (14). After electrophoresis, the gel was dried and autoradiographed. The lanes marked YCp88, pY176, pY1710, and pY11 refer to the plasmid source for the mRNA which was translated to result in the fusion proteins indicated at the top. (C) Autoradiograph of the gel mobility assay using various protein concentrations of fusion proteins. Aliquots (1 µl) of 1/1,000, 1/100, and 1/10 dilutions and of an undiluted sample of each translation mix were incubated with ^{32}P-labeled *lexA* operator and applied to a 5% polyacrylamide gel containing a low-ionic-strength buffer (14). After electrophoresis, the gel was dried and autoradiographed. The lanes marked YCp88 and pY1710 refer to the plasmid source for the mRNA which was translated to result in the fusion proteins indicated at the top; 0.001, 0.01, 0.1, and 1 refer to the dilution factors 1/1,000, 1/100, 1/10, and undiluted, respectively.

the fusion products. Most probably, because HMGs bind nonspecifically to DNA, their effective target is in a substantially higher concentration, thereby masking or inhibiting the specific binding of the LexA domain of the fusion proteins. In contrast, the C-terminal region of the HMG-17 protein, which lacks a DNA-binding domain, when fused to a LexA DNA-binding domain, does not inhibit the LexA region from binding to the *lexA* operator DNA. We conclude that the LexA–HMG-17/C fusion protein can indeed bind specifically to the *lexA* operator. Similar results would be expected with the HMG-14 constructs because of the high degree of structural homology between the two proteins. Likewise, construct pY15 lacks the HMG-1 DNA-binding domain and is similar to other fusion constructs in which the LexA DNA-binding domain is fused to a negatively charged polypeptide (18, 19, 30, 38). By analogy, it could be expected that the fusion protein product of this construct would similarly bind its target. As shown in Fig. 5C, lower absolute amounts of fusion proteins shifted only a fraction of the labeled oligonucleotide. It is also possible that the binding of these fusion proteins could be different in vitro and in vivo as a result of the differences in absolute concentrations of these proteins in the binding assay and in chromatin.

β-Galactosidase activity in yeast cells expressing LexA-HMG fusion proteins. Assays of β-galactosidase activities of LexA-HMG fusion proteins indicated that HMG-14, HMG-17, and HMG-1 polypeptide chains and the various peptides derived from them are not capable of activating transcription in a yeast system from a reporter plasmid containing the β-galactosidase sequence downstream from a *lexA* operator (Table 1). Yeast cells were cotransfected with the reporter plasmid and the various LexA constructs described above. Analysis of the β-galactosidase activities present in the extracts prepared from these cells indicated that the LexA-HMG fusion proteins, as well as the negative control, produced negligible activity, from 0.5 to 3.9 U of β-galactosidase. Thus, neither the complete HMG proteins nor deletion mutants containing progressively higher negative-charge densities yielded detectable increases in β-galactosidase activity. In contrast, similar extracts from cells expressing the positive control, the LexA-GCN4 fusion product, yielded 304.9 U of β-galactosidase activity. Although data for pY1710 were not obtained, the close homology between the primary structures of HMG-14 and HMG-17 suggests that similar results would be expected. In fact, the C-terminal region of HMG-14 has an even higher degree of negative charge than does that of HMG-17.

DISCUSSION

The association of both classes of HMG proteins (i.e., the HMG-14/-17 and HMG-1/-2 classes) with actively transcribed regions of the chromatin (13, 16, 45, 47, 49–51) has led to the hypothesis that these proteins play a functional role in the transcribability of genes. It is possible that these proteins are structural proteins which alter the conformation of a nucleosomal subset, thereby allowing transcription

TABLE 1. β-Galactosidase activities of yeast cells containing plasmids expressing LexA-HMG fusion proteins

Plasmid	β-galactosidase activity (U)[a]
YCp88-gcn4Δ19	304.9
pY176	2.6
pY177	1.3
pY1710	ND
pY142	2.6
pY143	1.1
pY146	0.7
pY11	3.9
pY12	1.4
pY15	0.5
pX	1.4

[a] Calculated as described by Guarente (17). Each value is an average of six A_{420} readings. ND, not determined.

factors to identify different chromatin regions. However, it is also possible that the HMG proteins can play an active role in gene regulation by functioning as quasi transcription factors. The structural analyses presented here could favor the latter possibility. The protein sequences are highly conserved, and consequently the charge distributions are similarly conserved. Analysis of the structures of the human and chicken HMG-14 and HMG-17 reveals that they are related molecules with a common ancestor (26). The N termini are highly charged, with a net positive charge, and the DNA-binding domains are almost identical (1, 4) and are extremely basic, as are the proximal C-terminal peptides following the DNA-binding domains. The C-terminal thirds of both HMG-14 and -17 are significantly more acidic. These analyses demonstrate structural similarities between HMG proteins and transcription factors having negatively charged activating domains (10, 34, 36, 41, 44).

In this study we tested whether HMGs can serve as transcription activators in a fashion analogous to that of other transcription activators containing negatively charged activating domains. These experiments demonstrated that the transcription-activating domains in these proteins could be localized to an acidic region (10, 34, 36, 41, 44). These regions could act independently of the rest of the protein. In an analogous fashion, we have tested the negatively charged domains of HMG proteins. Our data show that the negatively charged HMG domain does not interfere with the binding of the LexA fusion protein to its operator. The data clearly show that HMG proteins cannot serve as transcription factors in the same manner as can GCN4 (18, 19) and GAL4 (30), whose potential to activate transcription seems to be dependent, at least in part, on the presence of a negatively charged, amphipathic α helix. HMG-14 and HMG-17 could indeed form a negatively charged α helix. However, these helical regions are not amphipathic. It is not clear whether HMG-14 and HMG-17, in their native state, contain a negatively charged surface. However, induced α-helical structures could be stabilized by other intranucleosomal interactions, thus exposing a negatively charged protein veneer on a subset of nucleosomes containing HMG proteins.

Obviously, the relative amounts of HMG-14 and HMG-17 in the nuclei of higher eucaryotes are such that if these proteins indeed did function as transcription activators such as VP16, GAL4, and GCN4, most genes would be constitutively transcribed. In spite of this, we have tested these proteins for their potential to activate transcription because there is evidence that they play a functional role in transcription and because there are structural similarities between HMGs and some transcription factors. The data here suggest that in this test system, HMG-14, HMG-17, and HMG-1 do not function as transcription activators. If they are indeed part of the transcription apparatus, then their effects are different from those of the better-studied transcription factors.

ACKNOWLEDGMENTS

We thank Kevin Struhl for the yeast strain and plasmids YCp88-lexA-gcn4Δ19 and YEp21-Sc3423, John Little for the anti-LexA antibody, and Mark Boguski and David States for reviewing the manuscript.

REFERENCES

1. **Abercrombie, B. D., G. G. Kneale, C. Crane-Robinson, E. M. Bradbury, G. H. Goodwin, J. M. Walker, and E. W. Johns.** 1978. Studies on the conformational properties of the high-mobility-group chromosomal protein HMG 17 and its interaction with DNA. Eur. J. Biochem. **84**:173–177.
2. **Bustin, M., D. A. Lehn, and D. Landsman.** 1990. Structural features of the HMG chromosomal proteins and their genes. Biochim. Biophys. Acta **1049**:231–243.
3. **Carballo, M., P. Puigdomenech, and J. Palau.** 1983. DNA and histone H1 interact with different domains of HMG 1 and 2 proteins. EMBO J. **2**:1759–1764.
4. **Cary, P. D., D. S. King, C. Crane-Robinson, E. M. Bradbury, A. Rabbani, G. H. Goodwin, and E. W. Johns.** 1980. Structural studies on two high-mobility-group proteins from calf thymus, HMG-14 and HMG-20 (ubiquitin), and their interaction with DNA. Eur. J. Biochem. **112**:577–580.
5. **Chou, P. Y., and G. D. Fasman.** 1978. Prediction of the secondary structure of proteins from their amino acid sequence. Adv. Enzymol. **47**:45–148.
6. **Dodgson, J. B., D. L. Browne, and A. J. Black.** 1988. Chicken chromosomal protein HMG-14 and HMG-17 cDNA clones: isolation, characterization and sequence comparison. Gene **63**: 287–295.
7. **Dorbic, T., and B. Wittig.** 1986. Isolation of oligonucleosomes from active chromatin using HMG17-specific monoclonal antibodies. Nucleic Acids Res. **14**:3363–3376.
8. **Dorbic, T., and B. Wittig.** 1987. Chromatin from transcribed genes contains HMG17 only downstream from the starting point of transcription. EMBO J. **6**:2393–2399.
9. **Druckmann, S., E. Mendelson, D. Landsman, and M. Bustin.** 1986. Immunofractionation of DNA sequences associated with HMG-17 in chromatin. Exp. Cell Res. **166**:486–496.
10. **Earnshaw, W. C.** 1987. Anionic regions in nuclear proteins. J. Cell Biol. **105**:1479–1482.
11. **Ebina, Y., Y. Takahara, F. Kishi, A. Nakazawa, and R. Brent.** 1983. LexA protein is a repressor of the colicin E1 gene. J. Biol. Chem. **258**:13258–13261.
12. **Einck, L., and M. Bustin.** 1983. Inhibition of transcription in somatic cells by microinjection of antibodies to chromosomal proteins. Proc. Natl. Acad. Sci. USA **80**:6735–6739.
13. **Einck, L., and M. Bustin.** 1985. The intracellular distribution and function of the high mobility group chromosomal proteins. Exp. Cell Res. **156**:295–310.
14. **Fried, M., and D. M. Crothers.** 1981. Equilibria and kinetics of lac repressor-operator interactions by polyacrylamide gel electrophoresis. Nucleic Acids Res. **9**:6505–6525.
15. **Garnier, J., D. J. Osguthorpe, and B. Robson.** 1978. Analysis of the accuracy and implications of simple methods for predicting the secondary structure of globular proteins. J. Mol. Biol. **120**:97–120.
16. **Goodwin, G. H., and C. G. P. Mathew.** 1982. Role in gene structure and function, p. 193–221. *In* E. W. Johns (ed.), The HMG chromosomal proteins. Academic Press, Orlando, Fla.
17. **Guarente, L.** 1983. Yeast promoters and lacZ fusions designed to study expression of cloned genes in yeast. Methods Enzymol. **101**:181–191.
18. **Hope, I. A., S. Mahadevan, and K. Struhl.** 1988. Structural and functional characterization of the short acidic transcriptional activation region of yeast GCN4 protein. Nature (London) **333**:635–640.
19. **Hope, I. A., and K. Struhl.** 1986. Functional dissection of a eukaryotic transcriptional activator protein, GCN4 of yeast. Cell **46**:885–894.
20. **Hurstel, S., M. Granger-Schnarr, and M. Schnarr.** 1988. Contacts between the lexA repressor—or its DNA-binding domain—and the backbone of the *recA* operator DNA. EMBO J. **7**:269–275.
21. **Igo-Kemenes, T., W. Horz, and H. G. Zachau.** 1982. Chromatin. Annu. Rev. Biochem. **51**:89–121.
22. **Ito, H., Y. Fukuda, K. Murata, and A. Kimura.** 1983. Transformation of intact yeast cells treated with alkali cations. J. Bacteriol. **153**:163–168.
23. **Kaplan, D. J., and C. H. Duncan.** 1988. Full length cDNA sequence for bovine high mobility group 1 (HMG1) protein. Nucleic Acids Res. **16**:10375.
24. **Kleinschmidt, J. A., U. Scheer, M. C. Dabauvalle, M. Bustin,**

and W. W. Franke. 1983. High mobility group proteins of amphibian oocytes: a large storage pool of a soluble high mobility group-1-like protein and involvement in transcriptional events. J. Cell Biol. **97**:838–848.

25. **Laemmli, U. K.** 1970. Cleavage of structural proteins during the assembly of the head of bacteriophage T4. Nature (London) **227**:680–685.
26. **Landsman, D., and M. Bustin.** 1986. Chromosomal proteins HMG-14 and HMG-17: distinct multigene families coding for similar types of transcripts. J. Biol. Chem. **261**:16087–16091.
27. **Landsman, D., N. Soares, F. J. Gonzalez, and M. Bustin.** 1986. Chromosomal protein HMG-17: complete cDNA sequence and evidence for a multigene family. J. Biol. Chem. **261**:7479–7484.
28. **Landsman, D., T. Srikantha, R. Westermann, and M. Bustin.** 1986. Chromosomal protein HMG-14: complete cDNA sequence and evidence for a multigene family. J. Biol. Chem. **261**:16082–16086.
29. **Landsman, D., S. Zavou, N. Soares, G. H. Goodwin, and M. Bustin.** 1988. Mouse non-histone chromosomal protein HMG-17 cDNA sequence. Nucleic Acids Res. **16**:10386.
30. **Ma, J., and M. Ptashne.** 1987. Deletion analysis of GAL4 defines two transcriptional activating segments. Cell **48**:847–853.
31. **Malik, N., M. Smulson, and M. Bustin.** 1984. Enrichment of acetylated histones in polynucleosomes containing high mobility group protein 17 revealed by immunoaffinity chromatography. J. Biol. Chem. **259**:699–702.
32. **Maniatis, T., E. F. Fritsch, and J. Sambrook.** 1982. Molecular cloning: a laboratory manual. Cold Spring Harbor Laboratory, Cold Spring Harbor, N.Y.
33. **Mardian, J. K., A. E. Paton, G. J. Bunick, and D. E. Olins.** 1980. Nucleosome cores have two specific binding sites for nonhistone chromosomal proteins HMG 14 and HMG 17. Science **26**:1534–1536.
34. **Mitchell, P. J., and R. Tjian.** 1989. Transcriptional regulation in mammalian cells by sequence-specific DNA binding proteins. Science **245**:371–378.
35. **Paonessa, G., R. Frank, and R. Cortese.** 1987. Nucleotide sequence of rat liver HMG1 cDNA. Nucleic Acids Res. **15**:9077.
36. **Ptashne, M.** 1988. How eukaryotic transcriptional activators work. Nature (London) **335**:683–689.
37. **Reeves, R.** 1984. Transcriptionally active chromatin. Biochim. Biophys. Acta **782**:343–393.
38. **Sadowski, I., J. Ma, S. Triezenberg, and M. Ptashne.** 1988. GAL4-VP16 is an unusually potent transcriptional activator. Nature (London) **335**:563–564.
39. **Sandeen, G., W. I. Wood, and G. Felsenfeld.** 1980. The interaction of high mobility proteins HMG14 and 17 with nucleosomes. Nucleic Acids Res. **8**:3757–3778.
40. **Shick, V. V., A. V. Belyavsky, and A. D. Mirzabekov.** 1985. Primary organization of nucleosomes. Interaction of non-histone high mobility group proteins 14 and 17 with nucleosomes, as revealed by DNA-protein crosslinking and immunoaffinity isolation. J. Mol. Biol. **185**:329–339.
41. **Sigler, P. B.** 1988. Acid blobs and negative noodles. Nature (London) **333**:210–212.
42. **Singh, J., and G. H. Dixon.** 1990. High mobility group proteins 1 and 2 function as general class II transcription factors. Biochem. **29**:6295–9302.
43. **Srikantha, T., D. Landsman, and M. Bustin.** 1988. Cloning of the chicken chromosomal protein HMG-14 cDNA reveals a unique protein with a conserved DNA binding domain. J. Biol. Chem. **263**:13500–13503.
44. **Struhl, K.** 1987. Promoters, activator proteins, and the mechanism of transcriptional initiation in yeast. Cell **49**:295–297.
45. **Tremethick, D. J., and P. L. Molloy.** 1988. Effects of high mobility group proteins 1 and 2 on initiation and elongation of specific transcription by RNA polymerase II in vitro. Nucleic Acids Res. **16**:11107–11123.
46. **Walker, J. M.** 1982. Primary structures, p. 69–87. *In* E. W. Johns (ed.), The HMG chromosomal proteins. Academic Press, Orlando, Fla.
47. **Watt, F., and P. L. Molloy.** 1988. High mobility group proteins 1 and 2 stimulate binding of specific transcription factor to the adenovirus major late promoter. Nucleic Acids Res. **16**:1471–1485.
48. **Weintraub, H., and M. Groudine.** 1976. Chromosomal subunits in active genes have an altered conformation. Science **193**:848–856.
49. **Weisbrod, S.** 1982. Active chromatin. Nature (London) **297**:289–295.
50. **Weisbrod, S.** 1982. Properties of active nucleosomes as revealed by HMG 14 and HMG 17 chromatography. Nucleic Acids Res. **10**:2017–2042.
51. **Weisbrod, S., M. Groudine, and H. Weintraub.** 1980. Interaction of HMG 14 and 17 with actively transcribed genes. Cell **19**:289–301.
52. **Wen, L., J.-K. Huang, B. H. Johnson, and G. R. Reeck.** 1989. A human placental cDNA clone that encodes nonhistone chromosomal protein HMG-1. Nucleic Acids Res. **17**:1197–1214.
53. **Westermann, R., and U. Grossbach.** 1984. Localization of nuclear proteins related to high mobility group protein 14 (HMG 14) in polytene chromosomes. Chromosoma **90**:355–365.