Recombinant human chromosomal proteins HMG-14 and HMG-17

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ABSTRACT

Vectors for expressing human chromosomal proteins HMG-14 and HMG-17 in bacterial cultures under the control of the temperature-inducible λ P_L promoter have been constructed. The open reading frames of the cDNAs have been amplified by the polymerase chain reaction (PCR), utilizing amplimers containing desired restriction sites, thereby facilitating precise location of the initiation codon downstream from a ribosomal binding site. Expression of the recombinant proteins does not significantly affect bacterial growth. The rate of synthesis of the recombinant proteins is maximal during the initial stages of induction and slows down appreciably with time. After an initial burst of protein synthesis, the level of the recombinant protein in the bacterial extracts remains constant at different times following induction. Methods for rapid extraction and purification of the recombinant proteins are described. The recombinant proteins are compared to the proteins isolated from eucaryotic cells by electrophoretic mobility, Western analysis and nucleosome core mobility-shift assays. The ability of the proteins to shift the mobility of the nucleosome cores, but not that of DNA, can be used as a functional assay for these HMG proteins. A source for large quantities of human chromosomal proteins HMG-14 and HMG-17 will facilitate studies on their structure, cellular function and mechanism of interaction with nucleosomes.

INTRODUCTION

Chromosomal proteins HMG-14 and HMG-17 are among the most abundant, ubiquitous and evolutionary conserved nonhistone proteins present in the nuclei of higher eucaryotes (1). The two proteins bind to the inner side of the nucleosomal DNA (2) potentially altering certain interactions between the DNA and the histone octamer. Each nucleosome has two binding sites for the proteins (3−5, 36). However, the limited amount of protein in the nucleus (5) confines their presence to about 10% of the nucleosomes. The binding of the proteins to nucleosomes is salt dependent. At low ionic strength the entire molecule is bound to the nucleosome (7) and the binding is noncooperative. At higher ionic strength the binding is cooperative (8, 9) and the molecule is bound only via its central, positively charged domain (7). Immunochemical analysis of the exposure of HMG surfaces in chromatin reveals that the DNA binding domain is exposed to a lesser degree than the negatively charged, C-terminal domain of the protein. The C-terminal domain is free to interact with other molecules (10). The binding of HMG-14 and HMG-17 to nucleosomes stabilizes the core particle (11), increases its radius of gyration (12) and affects its digestibility by DNaseI (13). The length of the nucleosomal repeat in chromatin affects the binding of the proteins (14). Recently, Graziano and Ramakrishnan reported that the nucleosome density in chromatin fibers containing HMG-14 is lower than that of HMG-depleted chromatin (5).

Both HMG-14 and HMG-17 have a modular structure and an uneven distribution of charged residues along the polypeptide chain (1). The N-terminal region of the molecules has a slight positive charge, the central region, which contains the DNA binding domain, has a high positive charge and the C-terminal region of the molecules has a net negative charge. Furthermore, helical wheel projections of these negatively charged C-terminal regions indicate that the negative charged residues are clustered on one surface of the helix. These features are similar to those found in certain transcriptional activators (15). The structure of the proteins, their mode of binding to nucleosomes and their effect on nucleosome density are consistent with a role in transcription. Results obtained from nuclease digestion (16), nucleosome reconstitution (4, 17), immunofluorescence (18), immunofractionation (19−21) and antibody microinjection (22) experiments are consistent with the proposal that the two proteins may be involved in a process which confers distinct properties to chromatin regions containing transcribable genes (23). However, in spite of these studies, the detailed cellular function of the proteins is not known.

Studies on the structure of the proteins, their binding to chromatin and nucleosomes and their cellular function require a readily available source for these molecules. We have isolated and characterized the cDNAs and genes for both HMG-14 and HMG-17 from several species (1). Here we describe the

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utilization of bacterial expression vectors and the cDNAs for human HMG-14 (24) and human HMG-17 (25), for the synthesis of large quantities of these proteins.

MATERIALS AND METHODS

Materials

LB media was from Digene, enzymes and buffers from Boehringer Mannheim and BRL, T4 DNA ligase and Klenow fragment of DNA polymerase from New England Biolabs, agarose from FMC.

Cells and plasmids

The *E. coli* strain RRI, containing the low copy number plasmid pRK248cIts, which carries the temperature sensitive cI repressor gene (26), was used to propagate plasmid pRC23, as described (27). The expression vector pRC23 is a derivative of pBR322 that contains the phage λ P_L promoter and a consensus ribosomal binding site (28) located 150 nucleotides downstream from the start of transcription (27). The expression vector pRC23Δ is a derivative of pRC23 containing a deletion of ~600 bp between the BalI and PvuII sites in the pBR322 sequence area. Both vectors were designed to express heterologous genes that have been modified at the 5′ end so that an ATG initiation codon is adjacent to an EcoRI cleavage site. The EcoRI site in these plasmids is located at the 3′ end of the SD (Shine and Dalgarno) sequence.

Construction of p14

The open reading frame (ORF) contained in the human HMG-14 cDNA (24) was amplified by PCR. The 5′ amplimer is a 29-mer with the sequence 5′TAATGAATTCCCAGATGCCCAAGA-**GGAAG**3′. The nucleotides shown in bold characters are complementary to the non-coding strand at the start of translation. An EcoRI recognition site (underlined) was placed 11 nucleotides upstream from the ATG. The 3′ amplimer is a 25-mer: 3′**CTAATTATT**GGTATATGGTTTCGAA5′ and was designed to be complementary to the coding strand at the 3′ end of the ORF (nucleotides shown in bold letters). A HindIII recognition site (underlined) was placed 18 nucleotides downstream from the TAA termination codon. Amplification by PCR of the cDNA with these amplimers yielded a DNA fragment of 0.33 kb, containing the open reading frame for HMG-14, flanked by EcoRI and HindIII sites at its 5′ and 3′ ends respectively. This fragment was purified from the amplimers on low melting agarose gel and extracted from the gel through elutip-d (S&S) following the procedures provided by the manufacturer. The purified fragment was blunt-end ligated into pRC23 at the EcoRI site, which was previously filled-in with Klenow fragment. The ligation mixture was used to transform *E. coli* RRI. Plasmid transformation procedures were performed as described (29). Transformants were selected on L-agar plates containing both ampicillin and tetracycline and the presence of inserts was confirmed by restriction analyses with EcoRI and HindIII. One transformant out of 8 had insert in the correct orientation and was named plasmid p14. This construct had the HMG-14 ORF under the control of the P_L promoter and its ATG was 19 nucleotides 3′ from the SD region (see Fig. 1). The HMG-14 ORF was also subcloned into Phagescript SK (Stratagene) and sequenced using the Sequenase kit and protocols supplied by United States Biochemical.

Construction of p17

The open reading frame of human HMG-17 cDNA (25) was amplified by PCR. The 5′ amplimer, 5′TAATGAATTC**ACCA-TGCCCAAGAGAAAG**3′, is 28 bp long and contains an EcoRI recognition site (underlined) 10 nucleotides upstream from the ATG codon. The 3′ amplimer, 3′**GGTTCACTT**CACAC-ACGTTTCGAA5′, contains a HindIII recognition site (underlined) placed 14 nucleotides downstream from the TGA termination codon. The PCR product is a DNA fragment of 304 bp containing the open-reading frame for HMG-17 flanked by EcoRI and HindIII sites at its 5′ and 3′ ends, respectively. This DNA was purified, restricted with EcoRI and HindIII and cloned into pBluescript II (Stratagene) at its EcoRI and HindIII sites (See Fig. 2). The HMG-17 ORF was then subcloned into Phagescript SK+ (Stratagene) and sequenced using the Sequenase kit (United States Biochemical). The HMG-17 ORF was excised from pBluescript II and ligated into pRC23Δ at its EcoRI and HindIII sites. The ligation reaction mixture was used to transform *E. coli* RRI [pRK248cIts] as described above. The transformation efficiency was 0.7 transformants per ng of pRC23Δ EcoRI/HindIII vector. Out of 12 transformants 10 contained the ORF-17 insert, as assayed by restriction digestion of their plasmids DNA with EcoRI and HindIII. A clone was selected and named p17. This construct has the HMG-17 ORF under the control of the P_L promoter. The ATG codon is 10 nucleotides away from the SD sequence (see Figure 2).

Expression of HMG proteins

The transformed bacterial strains were grown, at 32°C, in LB containing 50 μg/ml ampicillin, to the desired optical density. The temperature was rapidly raised to 42°C by incubating the flask in a 65°C water bath, and the bacteria grown at 42°C for several hours. For analytical purposes, 100 ml cultures were grown to an OD_{600} of 0.2, shifted to 42°C and grown for

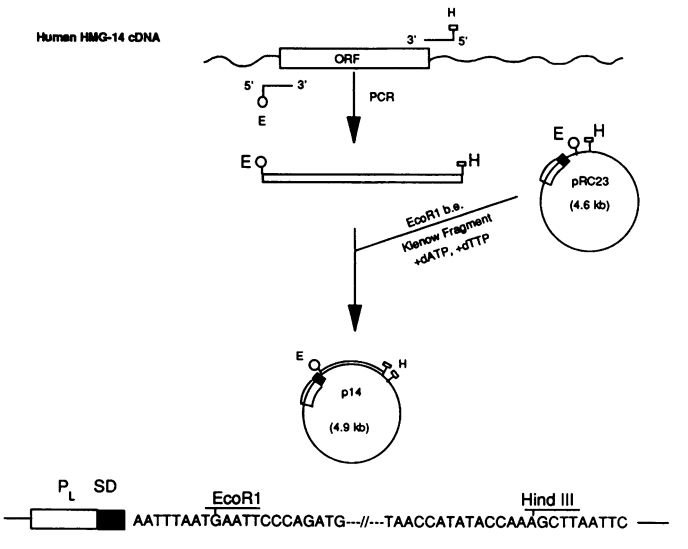

Figure 1. Construction of expression plasmids for human HMG-14. The open reading frame (ORF) present in the cDNA was amplified by PCR using amplimers containing restriction sites. The PCR product was purified and blunt end ligated into plasmid pRC23 which was restricted with EcoRI and blunt ended. The sequence at the 5′ and 3′ end of the insert is shown. The regions containing the λ P_L promoter and the Shine-Dalgarno (SD) sequence are depicted, respectively, as open and black boxes. For further details see Materials and Methods section.

additional 3 hours. The cells were pelleted at 4°C, washed once with 10 mM Tris-HCl, pH 7.5, 1 mM EDTA, 100 mM NaCl and the pellet used for the extraction of proteins. Large scale preparations were done from either 2 or 25 liter bacterial cultures grown (30) in either 5 or 50 liter fermenters (New Brunswick Scientific, Edison, NJ). The bacteria were grown in LB medium supplemented with 10 g/l K_2HPO_4, 10 mM $MgSO_4$, 10 g/l glucose and 1 m/l trace elements solution (31). The cultures were grown at 32°C to an OD_{600} of ~6.0, shifted to 42°C and grown at this temperature for 3 additional hours. The cultures were chilled to 10°C and the bacteria collected with a continuous-flow centrifuge (Sharpless, Westminster, PA). The yield was approximately 20 g of wet *E. coli* per liter of culture. The HMGs were extracted either from the fresh pellet or from frozen bacterial pellets stored at −80°C.

Preparation of HMG proteins

The pellets were sonicated in 5 volumes of 5% perchloric acid (PCA) at 4°C for 30 seconds. The sonicate was stirred, at 4°C, for 2 hours and centrifuged at 10,000×g for 10 minutes. The pellet was discarded and the supernatant made 25% in trichloroacetic acid by the addition of 100% trichloroacetic acid. The mixture was kept at 4°C for 2 hours and centrifuged as above. The pellet was dissolved in 0.3 M HCl. To the acidified solution 6.5 volumes of acetone were added and the mixture kept at −20°C for at least 6 hours. The precipitate was collected by centrifugation, washed twice with 9:1 acetone:0.1 M HCl, once with 90% acetone and dried. A slight modification of the above method was used to extract the proteins from the preparative bacterial pellets. The pellets were suspended in 5 volumes of H_2O and the bacteria broken in a French press. The slurry was made 5% in PCA by the addition of 60% PCA. The suspension was treated as described above. The pellet obtained after the first acetone precipitation was resuspended in 0.3 M HCl and centrifuged at 10,000×g for 10 minutes. The pellet was discarded and the proteins precipitated from the supernatant by the addition of 6.5 volumes of acetone. The pellet obtained from this acetone treatment was washed as described above.

Purification by FPLC

HMG-14 was purified on Mono Q HR 5/5 column (Pharmacia). Buffer A was 20 mM Tris-HCl pH 8.2, 50 mM NACl; buffer B was 20 mM Tris-HCl pH 8.2, 1 M NaCl. Approximately 250 μg protein, in 10 mM Tris-HCl, pH 8.0, 10 mM NaCl, were loaded onto the column pre-equilibrated with buffer A. The column was washed with 4 volumes of buffer A and the protein eluted with a linear gradient (from solution A to B) at a rate of 1 ml per minute. The amount of buffer B in the eluting buffer was increased at the rate of 1% per minute. The HMG-14 protein was eluted as a single peak at 6% buffer B. Larger quantities were loaded on Mono Q HR 10/10 and the chromatographic conditions were scaled up as recommended by Pharmacia.

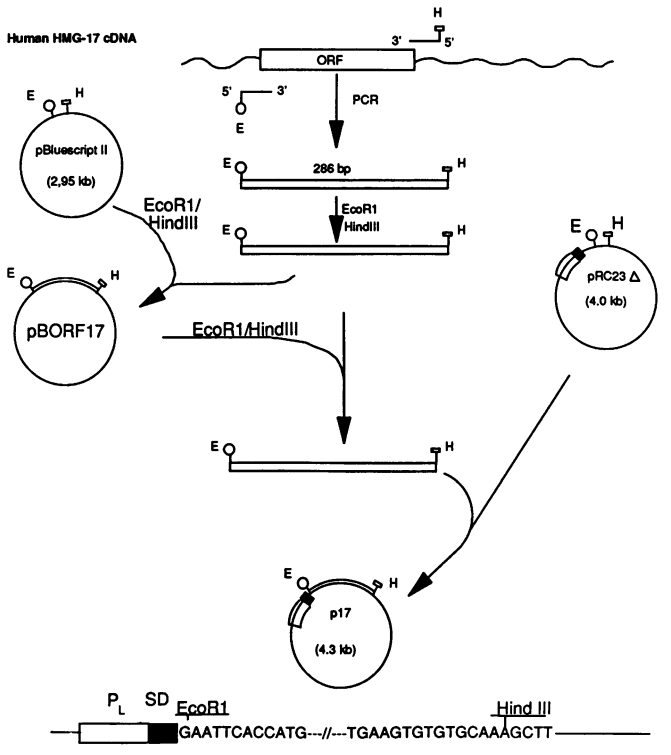

Figure 2. Construction of expression vectors for HMG-17. The open reading frame (ORF) present in the cDNA was amplified by PCR using amplimers containing restriction sites. The PCR product was purified, restricted with EcoRI and HindIII and subcloned into appropriately restricted pBluescript II to give plasmid pBORF17. The fragment was excised from this plasmid and cloned into pRC23Δ. The sequence at the 5′ and 3′ end of the insert is shown. The region containing the λ P_L promoter is depicted as an open box. The region containing the Shine-Dalgarno (SD) sequence is indicated. For further details see Materials and Methods section.

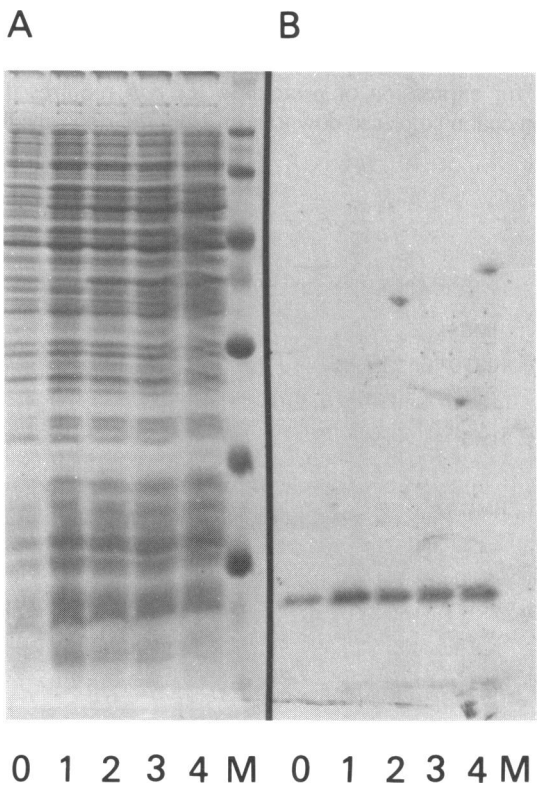

Figure 3. Expression of HMG-17. Bacterial cultures transformed with plasmed p17 were grown at 32°C to an OD_{600} of 0.2, shifted to 42°C and grown at this temperature for various periods of time. The cells were pelleted, washed once, sonicated and analyzed on an 8% polyacrylamide gel containing SDS. Each lane contained 40 μg protein. A, Coomassie blue stain of the gel; B, Western analysis with anti-HMG-17 antibodies. The numbers under the column indicate the time (hours) after shifting the cells to 42°C. M, molecular weight markers (high range, Gibco-BRL).

HMG-17 was purified on Mono S HR 5/5 columns using the same buffers and similar elution protocols. The purified protein eluted at 47% buffer B. The purity of the protein was assessed by electrophoresis in acid-urea and sodium dodecyl sulfate containing gels.

Nucleosome shift assay

Nucleosome core particles prepared from chicken erythrocytes according to Ausio *et al.* (32) were end-labelled (4) and incubated with HMG proteins in 20 mM Tris-HCl, pH 8.0, for 15 minutes on ice. The reaction mixture was applied to a 4% polyacrylamide gel and run under non-denaturing conditions, in 0.5×TBE (4). The gel was dried and autoradiographed.

Analytical techniques

Protein concentrations were determined with the BCA Protein Assay Kit (Pierce). Western analyses with antibodies to HMG-14 (18) or antibodies to HMG-17 (33) were done on Immobilon membranes (Millipore) as described previously (34). The procedure for electrophoresis in acid-urea containing gels or in sodium dodecyl sulfate containing gels have been described previously (35).

RESULTS AND DISCUSSION

Construction of vectors expressing HMG-14/-17 proteins

Construction of the expression vectors was facilitated by the availability of plasmids containing the full length cDNAs for both human HMG-14 and HMG-17 (24, 25). Efficient utilization of pRC23 for expression of proteins in *E. coli* requires that the initiation codon be placed downstream from the ribosome binding site (SD sequence) (27). The distance between the SD sequence and the initiation codon may affect the translation efficiency. We controlled the distance between the SD sequence and the initiation codon by using amplimers containing restriction sites, at the ends of the open reading frames, compatible with those present in the pRC23 vector (for details see Materials and Methods section). The PCR products obtained with these amplimers contain the open reading frames of either HMG-14, (see Fig. 1), or HMG-17 (see Fig. 2) flanked by EcoRI and HindIII sites at their 5′ and 3′ ends, respectively. The strategy for inserting the PCR products into the pRC23 vector differed slightly between the two constructs. For HMG-14 the PCR product was inserted into the single EcoRI site which was blunt ended. The orientation of the insert in the vector was tested by restriction analysis with EcoRI and HindIII. In p14, the ATG initiation codon is 19 nucleotides 3′ to the SD sequence. The ORF of HMG-17 was cloned into the vector which is previously restricted with both EcoRI and HindIII (see Figure 2). In the resulting plasmid, named p17, the ATG initiation codon is 9 nucleotides 3′ to the SD sequence. In this construct all the plasmids had the insert in the right orientation. To verify that the PCR products contained the open reading frames of HMG-14 and HMG-17, they were subcloned into Phagescript SK and sequenced. Analysis of the sequence verified that they contain the appropriate ORF sequences (data not shown).

Expression of HMG proteins

Initially, the expression of HMG proteins was tested in 100 ml bacterial cultures. The cultures were induced for expression at 42°C for various periods of time. The bacteria are sonicated,

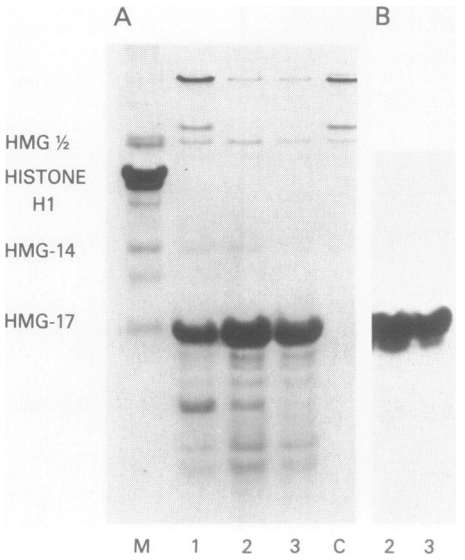

Figure 4. PCA extracts of bacterial cultures expressing HMG-17. Three cultures of *E. coli* RRI containing p17 were induced to express HMG-17 protein for 2 hours. The cells were harvested and the HMG-17 protein extracted with 5% PC-A, as detailed in the Materials and Methods section. The extract was analyzed on 20% acid-urea polyacrylamide gels. A, Coomassie blue stain; B, Western analysis with anti-HMG-17 antibodies. M, 5% PCA extract from human placenta used as marker proteins. The various proteins in the extract are identified on the left. Lanes 1, 2, 3, denote different clones expressing HMG-17. Lane C, 5% extract from a control bacterial culture which was transformed with the parental plasmid. The gels were purposely overloaded to show minor contaminants.

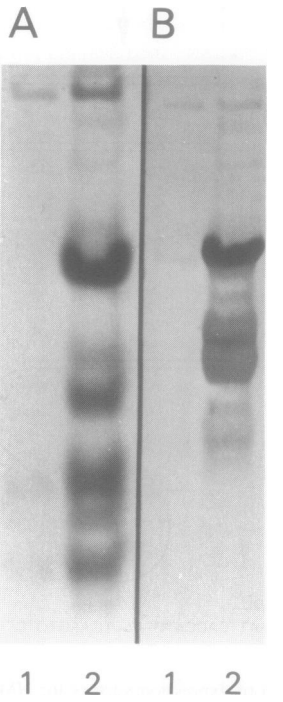

Figure 5. PCA extracts of bacterial cultures expressing HMG-14. A culture containing p14 was induced to express the protein and grown for 2 hours. The cells were harvested and the HMG protein extracted with 5% PCA. The extract was analyzed on 20% acid-urea polyacrylamide gels. A, Coomassie blue stain; B, Western analysis with anti-HMG-14 antibodies. 1, extract prepared from control bacterial cultures; 2, extract prepared from cells transformed with plasmid p14.

the proteins resolved by electrophoresis in SDS containing polyacrylamide gels and the expression of HMG detected by Western analysis. The data presented in Figure 3 depicts the expression of HMG-17 during a 4 hour growth period. In this experiment, at time 0, just prior to induction, HMG protein is detectable suggesting that the promoter may be leaky. The amount of HMG-17 slightly increases after growing the cells at 42°C for 1 hour. After this period the relative amount of HMG per bacteria seems to remain constant since the amount of protein detected after 1 hour is similar to that present after 4 hours. Although a gradual accumulation of HMG degradation products is expected, these were not detected. Conceivably, the putative degradation products are rapidly removed. Alternatively, there is an upper limit for the amount of HMG which a cell can express. Cultures expressing the HMG continue to grow and divide at approximately the same rate as the bacterial cultures containing the parental plasmid, therefore the HMG yield per liter of culture increases. This situation does not exist in all cases, since cells transformed with vectors expressing HIV-1 reverse transcriptase grow significantly slower than control cells transformed with parental plasmids (Becerra *et al.*, unpublished data).

Next, we tested whether the 5% PCA extraction procedure, developed for the preparation of HMG from eucaryotic sources, is suitable for the extraction of HMG proteins from bacteria. Figure 4 shows the 5% PCA extracts prepared from 3 different cell lines expressing HMG-17 and from a control cell line, containing the parental plasmid, grown under identical conditions. Each of the three p17 cultures contain a band with an electrophoretic mobility indistinguishable from that of HMG-17 isolated from human placenta. This band is not detectable in control cells. Western analysis with antibodies to HMG-17 confirms that the band is indeed HMG-17. To assess the purity of the preparation, the gels were overloaded so as to visualize minor contaminants. The transformed cells contain additional bands with a faster electrophoretic mobility than that of intact HMG-17. These bands, which constitute less than 10% of the main product, could be either degradation products of the HMG-17, peptides originating from premature chain termination or even bacterial proteins induced by the presence of HMG. They are not present in control cultures transformed with the parent plasmid.

Likewise, 5% PCA extracts of bacterial cultures transformed with plasmid p14 (Figure 5) contain a protein which reacts with antibodies elicited by calf thymus HMG-14. The culture contained additional bands which migrated faster than the intact protein suggesting that some of these may be either degradation products or products of premature chain termination.

Purification of proteins

Over 80% of the protein content of the 5% PCA extract is HMG. However, the preparations contain additional bands. Several of these are present in control bacterial cultures, indicating that they are not produced by the pRC23 constructs. Part of these bands could be eliminated from the preparation by changing the extraction protocol. The precipitate obtained with 25% trichloroacetic acid is suspended in 0.3 M HCl and insoluble material removed by centrifugation at 10,000×g for 10 minutes. This precipitate contains materials which are also present in control bacterial cultures. The HMG proteins were precipitated from the supernatant, as described in the methods section, by the addition of 6.5 volumes of acetone. Final purification was obtained by an FPLC step. HMG-14 was purified on Mono Q

Table I. Growth rate of bacterial cultures expressing human HMG-17

Time (hr)	OD_{600}	mg/ml* in PCA extract
0	6.6	0.2
0.5	12	n.d.
1	16	2.0
2	23	2.4
3	28	3.4
4	28	4.0

Cultures were grown at 32°C until the OD_{600} was 6.6 at which time the temperature was rapidly shifted to 42°C. *mg/ml protein was determined from the yield of the 5% PCA extract. n.d., not determined.

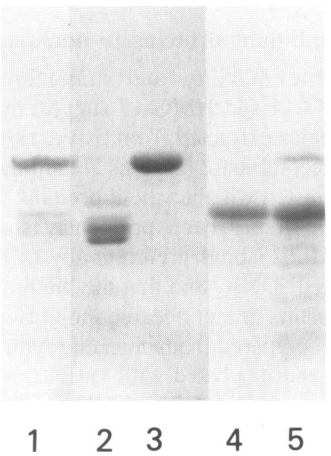

Figure 6. Purification of HMG by FPLC. HMG-14 was purified on Mono Q, HMG-17 on Mono S columns as described in the text. Fractions were collected and the proteins analyzed by electrophoresis in 18% polyacrylamide gels containing sodium dodecyl sulfate. 1, HMG-14 preparation applied to the column. 2, column flow-through containing mostly small molecular weight materials. 3, purified HMG-14. 5, purified HMG-17. 6, HMG-17 preparation applied to the column.

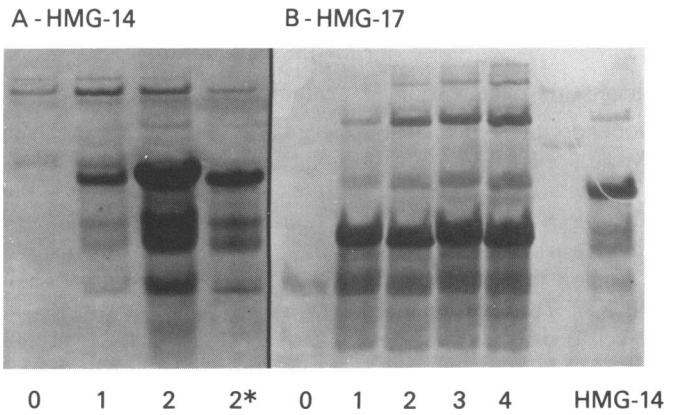

Figure 7. Stability of HMG proteins during bacterial growth. Aliquots were taken from bacterial cultures at various times after induction of HMG synthesis. The proteins soluble in 5% PCA were resolved by electrophoresis in acetic acid-urea gels. The gels were stained with Coomassie blue. The numbers at the bottom of the lanes indicate time after induction of protein synthesis, in hours. A, HMG-14. B, HMG-17. The cell pellets obtained from 50 ml bacterial cultures were dissolved in 200 μl buffer and 15 μl were applied to the gel except in column 2* where only 3 μl were applied. An HMG-14 marker is included in panel B. The gels were overloaded to show minor components in the preparation.

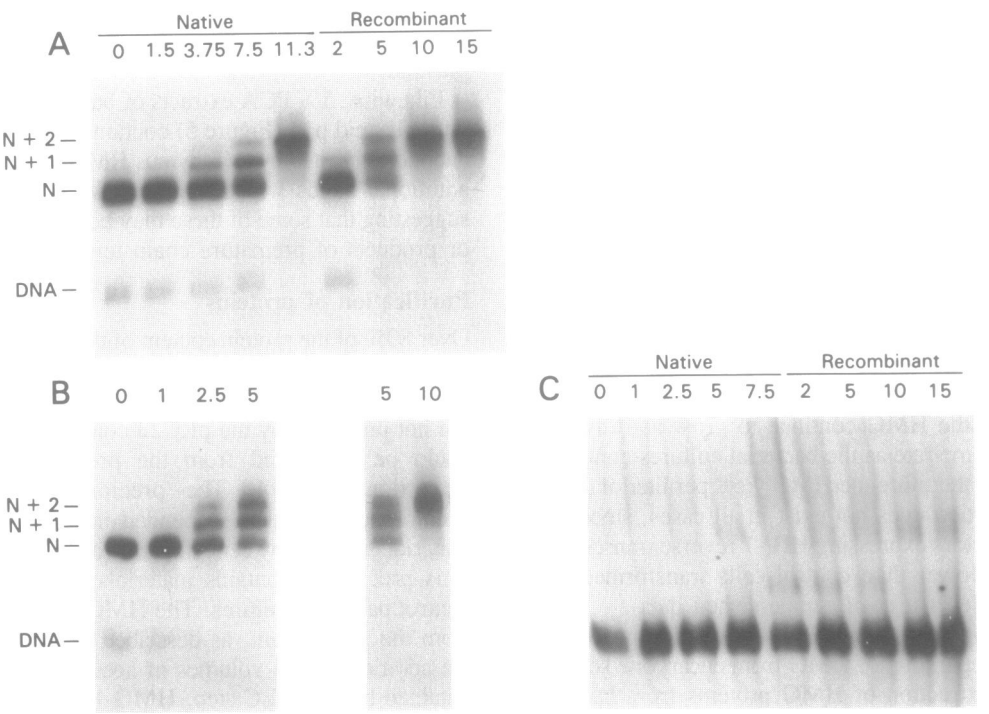

Figure 8. Binding of native and recombinant HMG-14 and HMG-17 protein to ^{32}P end-labelled nucleosomes and DNA. 32 ng of nucleosomes or DNA were incubated with various amounts of either native or recombinant protein. The incubation mixture was fractionated on native polyacrylamide gels as detailed in the methods section. A, HMG-17; B, HMG-14; C, DNA. The amount of protein (in ng) added is indicated on the top of the lanes. (5 ng HMG per 32 ng nucleosome cores corresponds to a molar ratio of 3.6:1). The migration of DNA, nucleosome cores (N) and cores with either one or two molecules of HMG (N+1 or N+2) is indicated on the left margin. Note that the proteins shifted the mobility of the nucleosomes but not that of the DNA.

column and eluted with Tris-HCl pH 8.0 buffer containing NaCl. As shown in Figure 6, the small-molecular weight materials eluted in the flow-through of the column and the HMG-14 protein is over 95% pure as determined by densitometry. For HMG-17, the extract was applied to a Mono S column which was eluted as described in the methods section. The HMG-17 fraction is 90% pure; it contains an additional protein band migrating just ahead of the main band (Figure 6). The final yield was approximately 5 mg protein per liter of bacterial culture.

Large-scale preparation of proteins

Pilot experiments for large-scale growth of bacteria expressing HMG-14 or HMG-17 were done in 5 liter fermenters as described in the methods section. The cultures were grown at 32°C to an $OD6_{600}$ of about 6.0 and shifted rapidly to 42°C. Aliquots were taken at various time points and the cells extracted with 5% PC-A. Table I presents the growth curve of the culture expressing HMG-17. The cells continued to divide for 3 hours at which point the cell density reached a maximum. The yield of the protein in the 5% PCA extract increases with time. Analysis of the 5% PCA extracts on acid-urea gels (Figure 7B) indicates that most of the HMG protein is synthesized within the first hour of induction and that the composition of the bacterial extract grown for 1 hour is very similar to that obtained from cells grown for 4 hours. In each case the main band corresponded to authentic HMG-17 (see Figure 4). The ratio of the main band to the faster migrating bands did not change, suggesting that longer growth does not increase protein degradation or the frequency of premature chain termination. Additional bands, with a molecular weight significantly larger than HMG-17, are also present in the preparations. The 2 liter culture yielded approximately 110 g of bacterial slurry which yielded 55 mg protein in the 5% PCA extract The large scale bacterial culture expressing HMG-14 also showed an increase in the total amount of protein without affecting the relative ratio of the intact HMG-14 to the other bands (see Figure 7A). The data suggest that there is a finite amount of HMG protein per cell and that the protein in the bacteria is relatively stable.

Binding of recombinant proteins to nucleosomes

The electrophoretic mobility and immunoreactivity of the recombinant HMG-14 and HMG-17 suggest that they are very similar to the proteins extracted from tissues or cells. To further test whether the recombinant proteins are similar to their native counterparts, we tested whether these proteins can interact with nucleosome cores. It has been previously shown (4, 8) that HMG-14 and HMG-17 bind preferentially to nucleosomes, as compared to 'naked' DNA, and that the binding of the proteins to nucleosomes results in a noticeable mobility shift (3–5, 36). Nucleosome cores prepared from chicken erythrocytes were end labelled with ^{32}P and incubated with various amounts of either native or recombinant HMG-14 or HMG-17. The incubation mixture was applied to 4% neutral polyacrylamide gels, dried and autoradiographed as detailed in the methods section. Addition of increasing amounts of HMG proteins lowered the mobility of a fraction of the nucleosome cores in increments until all the particles have a slower mobility (Figure 8). The incremental shift in nucleosome core mobility represent the binding of either one or two molecules of protein to the core particles (3, 4, 36). In contrast, addition of HMG proteins did not affect the mobility

of the 'naked' DNA (Figure 8C). The results obtained with the recombinant proteins are indistinguishable from those obtained with proteins extracted from tissues, suggesting that the recombinant proteins interact with DNA in a fashion similar to that of 'native' HMG proteins. The mobility shift may be used as a rapid and convenient assay for studies on the interaction of the proteins with nucleosome cores. Loss of preferential binding to nucleosomes, as compared to 'naked' DNA, resulting from mutation of residues in the proteins may point out which residues are important for specific nucleosome recognition. Thus, the recombinant proteins may facilitate studies on the structure and function of these abundant and ubiquitous nonhistone proteins.

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