Expression of Chromosomal Proteins HMG-14 and HMG-17 in Transformed Human Cells

Massimo P. Crippa, James M. Pash, Brenda I. Gerwin, Thomas E. Smithgall, Robert I. Glazer, and Michael Bustin

ABSTRACT

The relation between cellular phenotype and expression of chromosomal high mobility group proteins 14 and 17 (HMG-14 and HMG-17) has been examined in human cell lineages. Quantitation of HMG-14 and HMG-17 mRNA in several human cell lines revealed differences in both the steady state mRNA level and in the ratio of HMG-14 to HMG-17 mRNA. Analysis of phenotypically distinct derivatives of human bronchial epithelial cells revealed small differences between both the steady state mRNA levels and the relative amount of these proteins among the clonal variants. The effect of myeloid differentiation on the mRNA level of HMG-14 and HMG-17 was examined in the human promyelocytic leukemia cell line HL-60 following treatment with several granulocytic and monocytic differentiating agents. The ratio of HMG-17 mRNA to either HMG-14 or histone H4 mRNA varied among the cell phenotypes suggesting that phenotype switching may result in detectable alterations in the expression of the HMG-14 and HMG-17 genes. The data suggest that, although the ratio of HMG-14 to HMG-17 mRNA varies among human cell lines, these variations are relatively small.

INTRODUCTION

The HMG class of chromosomal proteins is one of the most abundant, ubiquitous, and evolutionarily conserved group of nonhistones found in the nuclei of all higher eukaryotes (1). Two members of this class, chromosomal proteins HMG-14 and HMG-17, may be involved in a process which maintains the chromatin structure of transcribable genes in a DNase I-sensitive conformation (2). In nucleosomes, the two proteins are positioned between the histone octamer and the DNA (3–6), thereby potentially affecting certain histone-DNA interactions. Although the cellular role of these proteins is not understood, data obtained from nuclease digestions (2, 7), nucleosome reconstitution (3, 4), immunofluorescence (8), immunofractionation (9–11), and antibody microinjection (12) are consistent with the proposal that the two proteins may be involved in the process which maintains transcribable genes in a unique chromatin conformation (7, 8).

The structure of the HMG/HMG gene family has been recently described (13–15); however, little is known about the transcriptional regulation of this gene family. We have noted that the mRNA for HMG-14/HMG-17 in HeLa cells is relatively abundant and that the levels fluctuate during the cell cycle, reaching maximum levels during the late S phase (16). The synthesis of the transcript is not coupled to DNA replication. It is not presently known whether the relative ratios of HMG-14 to HMG-17 are constant or differ in various cells. Phenotypic changes during cellular differentiation are often accompanied by changes in the expression of specific genes.

MATERIALS AND METHODS

Cell Culture. Bronchial epithelial cells were propagated in serum-free LHC-8 medium. For subculturing, cells were trypsin dissociated and inoculated into fibronectin/collagen-coated culture dishes. The sources, materials, and preparation of medium and solutions required for these procedures have been described (17–19). Human leukemia cell lines HL-60, U-937, THP-1, KG-1, and KG-1a were purchased from the American Type Culture Collection, Rockville, MD. All cells with the exception of KG-1 and KG-1a were maintained in RPMI 1640 medium containing 10% heat-inactivated fetal calf serum, 25 mm 4-(2-hydroxyethyl)-1-piperazineethanesulfonic acid, pH 7.4, 1 mm sodium pyruvate, nonessential amino acids, 100 units/ml penicillin, and 100 µg/ml streptomycin. KG-1 and KG-1a cells were grown in Iscove’s modified Dulbecco’s medium supplemented as described above. Cells were maintained at a density of 10³-10⁶ cells/ml. HL-60 cells were treated with the various differentiating agents (22) at the following concentrations: 1.6% (volume/volume) dimethyl sulfoxide, 1 µM retinoic acid, 0.1 µM 1,25-dihydroxyvitamin D₃, 1000 units/ml IFN-γ, and 10 nM TPA.

RNA Preparation, Slot Blot, and Northern Analysis. RNA was prepared and analyzed as described previously (23). Total cellular RNA was extracted from cell lines by the guanidinium thiocyanate method (24). For slot blot analysis, RNA was denatured in 3% formaldehyde–7× standard saline citrate at 65°C for 10 min and applied to Zetabind or Gene Screen Plus filters. For Northern analysis, RNA samples were treated with formaldehyde-formamide solutions and electrophoresed in denaturing formaldehyde gels (24). The gels were washed briefly with water and the RNA was nicked by short incubation with 0.2 n NaOH and transferred either to Zetabind or Gene Screen Plus by the Southern procedure (24). The Zetabind filters were treated as recommended by Church and Gilbert (25). The Gene Screen Plus filters were treated as recommended by the manufacturer.

DNA Probes. HMG-14 mRNA was detected with the purified insert excised from plasmid pH14c (26), which contains the entire human HMG-14 cDNA. HMG-17 mRNA was detected with the insert purified from plasmid pH17c (23), which contains the human HMG-17 cDNA, and human histone H4 mRNA was detected with the insert excised from plasmid p108A (27). The probes were labeled by nick translation (24).

Construction of Recombinant Plasmids. The full length cDNA for either human HMG-14 or HMG-17 was excised from plasmids pH14c (26) or pH17c (23), subcloned into pGEM-3Z vectors (Promega Biotech, and named pGEM14c and pGEM17c, respectively. Transcription from the T7 promoter of HaelIII-restricted pGEM14c gives a 247-
nucleotide probe for HMG-14 mRNA, while XbaI-restricted pGEM17c gives a 317-nucleotide probe for HMG-17 mRNA.

RNase Protection Assay. Samples of total RNA were precipitated with ethanol and redissolved in 30 μl hybridization buffer (40 mM piperazine-N,N′-bis(2-ethanesulfonic acid), pH 7.4–0.4 M NaCl-1.0 mM EDTA-80% formamide) containing 10° cpm of freshly prepared riboprobe. RNA was denatured by heating to 85°C for 5 min and hybridized for 16 h at 37°C. Samples were then digested with 350 μl RNase solution (10 mM Tris- HCl, pH 7.5–0.3 M NaCl-5 mM EDTA-40 μg/ml RNase A-2 μg/ml RNase T1) for 45 min at 30°C. RNase activity was quenched by adding 10 μl 20% sodium dodecyl sulfate and 2.5 μl (20 mg/ml) proteinase K and incubating for 15 min at 37°C. The reaction mixture was extracted with phenol:chloroform:isoamyl alcohol (25:24:1) and carrier RNA was added (10 μg), and the protected double-stranded RNA fragments were recovered by ethanol precipitation. The resulting pellets were dissolved in 10 μl loading buffer (80% formamide-1 mM EDTA-0.1% bromophenol blue-1% xylene cyanol) and resolved on denaturing 6% polyacrylamide/urea gels. Labeled protected fragments were visualized by autoradiography and quantitated by laser densitometry (26).

Protein Analysis. Cells were harvested by scraping into phosphate-buffered saline containing 1 mM phenylmethylsulfonyl fluoride and 0.1 mg/ml aprotinin (Sigma), followed by centrifugation at 500 × g. Nuclei were obtained from the cell pellets by homogenization in nuclei isolation buffer (10 mM Tris-HCl, pH 7.4–10 mM NaCl-3 mM MgCl2-0.5% Triton X 100), followed by centrifugation at 1600 × g. The nuclear pellets were washed once with nuclei isolation buffer, centrifuged, and resuspended in distilled water. Proteins were then extracted by treatment of nuclei with 5% HClO4, followed by centrifugation at 10,000 × g, and precipitation of the supernatant with 0.3 M HCl and 6 volumes of acetone. Proteins were separated by polyacrylamide gel electrophoresis and visualized by silver staining. Human HMG proteins were identified by comparing their migration with that of purified calf HMG-14/HMG-17 standards and by Western blot analysis using anti-HMG-14 (29) and anti-HMG-17 antibodies (30).

RESULTS

Expression of HMG-14/HMG-17 mRNA in Various Human Cells. To test whether the ratio of the two mRNAs differs between various human cells, the steady state mRNA levels from several myelomonocytic cell lines were analyzed by the RNase protection assay (Table 1, Fig. 1). The autoradiogram in Fig. 1 demonstrates the presence of both HMG-14 and HMG-17 mRNA in all cells tested.

Quantitation by laser densitometry (Table 1) indicated more than a 2-fold difference among the cells in the level of HMG-14 mRNA, from 1.3 units in THP-1 cells to 0.49 unit in HL-60 cells. The levels of HMG-17 mRNA varied less and ranged from 1.58 in KG-1 cells to 2.46 in U-937 cells. The ratio of HMG-17 to HMG-14 mRNA varied 3-fold from 4.9 for HL-60 cells. The levels of HMG-17 mRNA varied less and ranged from 1.3 units in THP-1 cells to 0.49 unit in HL-60 cells.

The data presented in Table 1 indicated that the HMG-17/HMG-14 mRNA ratio of KG-1 cells is identical to that of KG-1a cells. The KG-1a cell line is an undifferentiated subclone of KG-1 (22). The similarity in the ratio raises the possibility that within one particular cell lineage, or in closely related cells, the ratio of the two mRNAs is constant. Therefore, we examined clonal variants derived from 2 distinct cell types: normal human bronchial epithelial cells and HL-60 cells.

Cell Lines. Fig. 2 summarizes the clonal variants derived from normal human bronchial epithelial cells used in this study. The selection and properties of these derivatives have been described previously in detail (17–19). Briefly, transformation by Ad12-SV40 virus of primary human bronchial epithelial cells, obtained from explants of autopsy specimens, yielded cell line Beas-2B, which can be propagated in tissue culture and is weakly tumorigenic. Cell line S.6, which is induced to undergo terminal differentiation by serum, is a subclone of Beas-2B, while R.1 is a serum resistant line obtained from Beas-2B by serum selection. Both S.6 and R.1 cells are nontumorigenic. Infection of Beas-2B with Zip-vH-ras yielded BZR cells which are moderately tumorigenic. The highly tumorigenic cell line T33 was obtained by reestablishment, in tissue culture, of human cells from a tumor induced by BZR in an athymic nude mouse.

Fig. 3 summarizes the pathways of differentiation that HL-
60 cells undergo following treatment with various differentiating agents. Treatment with retinoic acid results in differentiation into granulocytic cells, treatment with either tumor necrosis factor, vitamin D3, or IFN-γ results in differentiation along the monocytic lineage, and treatment with TPA induces differentiation into macrophage-like cells (20-22).

Expression of HMG-14 and HMG-17 in Derivatives of Human Bronchial Epithelial Cells. Northern analysis of the HMG-14 and HMG-17 mRNA levels in various cells derived from human bronchial epithelial cells indicates that the steady state levels do not vary significantly among the cell types (Fig. 4). Because the levels of HMG-14 and HMG-17 mRNA fluctuate during the cell cycle, all cells were harvested below 80% confluence. The levels of histone H4 mRNA can be used to normalize the relative proportion of cells in the S phase of the cell cycle. From Fig. 2C, it is evident that the various cell types were at approximately the same stage of cell growth. From Table 2, it is evident that the fluctuations in HMG mRNA are relatively small. The ratio of HMG-17 to HMG-14 differed slightly among the various cells, suggesting that the HMG-14 and HMG-17 mRNA levels are not identical in all the clonal variants of human bronchial epithelial cells. Examination of a

<table>
<thead>
<tr>
<th>Cell line</th>
<th>HMG-14</th>
<th>HMG-17</th>
<th>HMG-17/HMG-14</th>
</tr>
</thead>
<tbody>
<tr>
<td>Beas-2B</td>
<td>2.01 (1.0)*</td>
<td>1.58 (1.0)</td>
<td>0.78 (1.0)</td>
</tr>
<tr>
<td>BZR</td>
<td>1.95 (1.0)</td>
<td>2.41 (1.5)</td>
<td>1.23 (1.6)</td>
</tr>
<tr>
<td>T33</td>
<td>2.36 (1.2)</td>
<td>2.19 (1.4)</td>
<td>0.92 (1.2)</td>
</tr>
<tr>
<td>S.6</td>
<td>2.52 (1.3)</td>
<td>1.68 (1.1)</td>
<td>0.66 (0.9)</td>
</tr>
<tr>
<td>R.1</td>
<td>1.36 (0.7)</td>
<td>2.84 (1.7)</td>
<td>2.08 (2.7)</td>
</tr>
</tbody>
</table>

* The values in parentheses represent relative amounts normalized to that found in Beas-2B cells. Data obtained as in Table 1.

Fig. 5. Northern analyses in various derivatives (Top) of HL-60 cells. A, HMG-14; B, HMG-17; C, histone H4. RNA was analyzed as described in "Materials and Methods" and in the legend to Fig. 4.

5% perchloric acid extract of purified nuclei indicated that the relative amount of the HMG proteins is similar throughout the entire lineage of the human bronchial epithelial cells (not shown). We conclude therefore that among the clonal subpopulations derived from human bronchial epithelial cells the steady state mRNA and the protein levels of both HMG-14 and HMG-17 are similar but not identical.

Expression of HMG-14 and HMG-17 in Derivatives of HL-60 Cells. Northern analyses of HMG-14, HMG-17, and histone H4 in phenotypic variants of HL-60 cells indicate variations in the steady state levels of these mRNAs (Fig. 5). The highest relative amount is present in rapidly dividing, log phase, HL-60 cells. Plateau phase or differentiated derivatives of HL-60 cells had significantly lower levels of both HMG-14 and HMG-17 mRNA. To quantify the relative levels of the different mRNAs, a slot blot analysis of the content of HMG-14, HMG-17, and histone H4 mRNA was performed (not shown). The ratio of HMG-17 to HMG-14 and of HMG-17 to H4 in the various cells are listed in Table 3. The HMG-17 to HMG-14 ratios ranged from 2.2 to 4.0. In monocytic like cells obtained by treatment of HL-60 cells with tumor necrosis factor, IFN-γ, or 1,25-dihydroxyvitamin D3, the ratio was 2.3 to 4.0, while in granulocytes produced by treatment with RA the ratio was 3.5. Log phase cells had a ratio of HMG-17/HMG-14 of 2.2, while plateau phase cells had a ratio of 3.6. Thus, the data do not suggest a correlation between the HMG-17 to HMG-14 ratio and the differentiation of HL-60 cells along either myeloid pathway. The ratio of HMG-17 to histone H4 also varied among the cells. This variation probably is due to the changes in histone H4 mRNA levels, a well-documented event in

Fig. 4. Northern analysis of HMG-14 and HMG-17 expression in derivatives of bronchial epithelial cells. Total cellular RNA was prepared from the cell lines indicated (Top), and 20-μg samples were electrophoresed on formaldehyde-containing agarose gels and transferred to Gene Screen Plus membranes as described in "Materials and Methods." Duplicate blots were prepared and probed for either HMG-14 or HMG-17 mRNA. One of the blots was reprobed with a histone H4 genomic probe. A, HMG-14 mRNA; B, HMG-17 mRNA; C, histone H4 mRNA; D, ethidium bromide stain of one of the gels. B2B, cell line Beas-2B.

Fig. 3. Differentiation pathway of HL-60 cells used in this study. See text for details.
Differentiating agents were slot blotted as described in the legend to Fig. 5. The 32P counts bound to each slot were determined by liquid scintillation.

With tumor necrosis factor.

Granulocytic or monocyctic phenotypes induced by treatment with either retinoic acid or IFN-γ, respectively. Northern analysis (Fig. 6) revealed slight variations in the mRNA levels during differentiation. The levels of mRNA were quantitated by slot blot analysis and the data obtained are shown in Fig. 7. The highest value obtained from the RNA extracted from the cells was designated as 100%. A, HMG-14; B, histone H4; C, HMG-17.

Fig. 6. Time course of HMG expression during HL-60 differentiation. RNA was extracted from either HL-60 cells or HL-60 cells treated with IFN-γ or retinoic acid for the days (Top). Duplicate Northern blots were prepared and probed for either HMG-14 or HMG-17 mRNA. One of the blots was reprobed for histone H4 (C). B and B1, different exposures of the HMG-17 blot.

Fig. 7. Quantitative changes in HMG-14/HMG-17 and histone H4 mRNA levels during HL-60 differentiation. Total cellular RNA, prepared from the cells described in Fig. 6, was slot blotted and analyzed with nick-translated probes. The 32P counts bound to each slot were determined by liquid scintillation. The highest value obtained from the RNA extracted from the cells was designated as 100%. A, HMG-14; B, histone H4; C, HMG-17.

growth-arrested cells. In spite of the relatively large fluctuation in cell cycle, the HMG-17 to HMG-14 mRNA ratios varies very little.

Next we tested whether the relative levels of the two HMG mRNAs change transiently during differentiation along the granulocytic or monocytic phenotypes induced by treatment with either retinoic acid or IFN-γ, respectively. Northern analysis (Fig. 6) revealed slight variations in the mRNA levels during differentiation. The levels of mRNA were quantitated by slot blot analysis and the data obtained are shown in Fig. 7. The highest level of the various mRNAs at any point in the culture are taken as 100%. The levels of both HMG-17 (Fig. 7C) and histone H4 (Fig. 7B) decreased on day 5 to approximately 50-70% of that detected on day 1 whether or not cells were treated with IFN-γ or retinoic acid. The levels of HMG-14 mRNA were more variable (Fig. 7A). Treatment with IFN-γ produced a change, relative to control, only on day 3, while retinoic acid produced an elevation only on day 3. Therefore, we conclude that treatment with IFN-γ or retinoic acid does not drastically change the steady state levels of HMG-17 mRNA but does cause variation in the level of HMG-14 mRNA. The changes in HMG-17 mRNA levels were very similar to those observed for histone H4 mRNA, suggesting that the changes observed are related to the cell cycle and not to differentiation per se. We have previously reported that in HeLa cells, HMG mRNA levels fluctuate throughout the cell cycle (16).

**DISCUSSION**

Previous studies indicated that chromosomal proteins HMG-14 and HMG-17 are present in all higher eukaryotes (1) and that they seem to be associated with transcriptionally active regions in the genome (2, 7, 8). Particularly noteworthy are the experiments of Westermann and Grossbach (29), which demonstrated that, in Chironomus pallidivittatus polytene chromosomes, HMG-14 is detectable at transcriptionally active puffs but is absent from the same loci prior to induction of transcription. This observation suggests that induction of gene activity may be associated with rearrangement in the chromosomal location of the HMG-14/HMG-17 proteins. The object of the present study was to examine whether changes in cell phenotype, which involve changes in gene expression, are accompanied by detectable changes in the cellular level of mRNA for chromosomal proteins HMG-14 or HMG-17.

The variants of the two cell lineages used in the present studies differ significantly from the parent cells. The derivatives of the immortalized human bronchial epithelial cells Beas-2B have distinct phenotypic characteristics. Two nontumorigenic clonal derivatives, S.6 and R.1, are either sensitive or resistant to induction of terminal squamous differentiation by serum or tumor growth factor-β. Likewise, the differentiated derivatives of HL-60 cells have distinct phenotypes which have been documented in detail elsewhere (20-22).

Our data indicate that the mRNA levels of HMG-14 and HMG-17 differ between various human myeloid cell lines (Fig. 1). Thus, a constant HMG-14 to HMG-17 mRNA ratio is not necessary for proper cell growth in tissue culture. We note, however, that the differences among the cell lines were relatively small and it is difficult to determine whether they are due to cell phenotype or to minor variations in the growth conditions.
of the different cell lines. Indeed, in the clonal variants of the human bronchial epithelial cells and in the two KG-1 clones, the mRNA levels varied little among the cells, suggesting that within a cell lineage the HMG-14/HMG-17 mRNA level is relatively constant. As indicated by the level of H4 mRNA, all the derivatives of these epithelial cells were harvested at approximately the same stage of the cell cycle, a prerequisite in these studies since the mRNA levels of the HMGs fluctuate throughout the cycle (16). The changes in the ratio of HMG-14 to HMG-17 mRNA among the phenotypic variants of HL-60 cells were of the same magnitude as those observed for variants of the bronchial epithelial cells and for human myeloid cell lines obtained from unrelated sources. Furthermore, the data were obtained by 3 different types of RNA analysis (DNase protection, Northern analysis, and slot blot quantitation), suggesting that the results were not dependent on the method of RNA analysis.

Developmental changes in transcriptional levels of genes coding for chromosomal proteins have not been studied in detail. The present study is the first investigation of the mRNA levels of the HMG-14/HMG-17 genes in various cells. Our data indicate that in immortalized cells capable of growing in tissue culture, the level of HMG-14/HMG-17 mRNA can vary as a function of cell growth. Within a cell lineage, the steady state mRNA levels are relatively constant provided that the growth conditions are tightly controlled. Several changes in phenotype were not accompanied by significant alterations in the ratio of HMG-17 to HMG-14 mRNA. The low variation in mRNA level is consistent with the notion that HMG-14 and HMG-17 are required components for cellular function in all higher eukaryotes. Indeed, we have noted that the genes have features typical of housekeeping genes (14, 15). However, it is still possible that in nonimmortalized cells, which have not been selected for the ability to grow in tissue culture, the mRNA level of these proteins vary.

ACKNOWLEDGMENTS

We wish to thank Dr. G. Stein for clone p108A and N. Soares for technical assistance.

REFERENCES

Expression of Chromosomal Proteins HMG-14 and HMG-17 in Transformed Human Cells

Massimo P. Crippa, James M. Pash, Brenda I. Gerwin, et al.


Updated version  Access the most recent version of this article at: http://cancerres.aacrjournals.org/content/50/7/2022

E-mail alerts  Sign up to receive free email-alerts related to this article or journal.
Reprints and Subscriptions  To order reprints of this article or to subscribe to the journal, contact the AACR Publications Department at pubs@aacr.org.
Permissions  To request permission to re-use all or part of this article, use this link http://cancerres.aacrjournals.org/content/50/7/2022. Click on "Request Permissions" which will take you to the Copyright Clearance Center's (CCC) Rightslink site.