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Selective decrease in low-M_r HMG proteins HMG I and HMG Y during differentiation of mouse teratocarcinoma cells

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We have studied the presence of high-mobility-group (HMG) chromatin proteins in undifferentiated F9 mouse teratocarcinoma cells and F9 cells, which were induced to differentiate by treatment with retinoic acid and dibutyryl-cAMP for 5 days. Acetic acid/urea-polyacrylamide gel electrophoresis and reversed-phase HPLC revealed that the induced F9 cells contained 77 and 62% less HMG I and HMG Y, respectively, than their untreated counterparts. The relative amounts of two other low-M_r HMG proteins HMG 14 and HMG 17 remained essentially unchanged and only a minor decrease was observed in the content of one of the high-M_r HMG proteins, HMG 2. The identity of the low-M_r HMG proteins was verified by amino acid analysis or partial sequencing. These results suggest that HMG I and HMG Y are HMG proteins specific for undifferentiated cells.

High-mobility-group protein; Cell differentiation: Chromatin; (Mouse teratocarcinoma cell)

1. INTRODUCTION

High-mobility-group proteins constitute a subclass of non-histone chromatin proteins of general occurrence in all four eukaryotic kingdoms [1]. The high-M_r HMG proteins HMG 1 and HMG 2 bind to single-stranded DNA and may affect the superhelicity of DNA [2,3]. The low-M_r HMG proteins HMG 14 and HMG 17 bind to DNA in nucleosomal core particles on the inner side of the DNA [4]. These proteins have been implicated in modifying the chromatin structure of transcriptionally active genes [5]. In addition to these well-characterized HMG proteins, two new low-M_r HMG proteins HMG I and HMG Y have been recently found from proliferating cells [6].

Mouse teratocarcinoma cell line F9 can be induced to differentiate to a cell type characteristic of parietal endoderm when treated with retinoic acid and dibutyryl-cAMP [7,8]. Since the available data suggest that HMG proteins are important structural elements of chromatin, we have studied the presence of these proteins in undifferentiated and differentiated F9 cells to elucidate further their role in the differentiation process.

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Abbreviations: HPLC, high-performance liquid chromatography; dibutyryl-cAMP, dibutyryladenosine 3',5'-cyclic monophosphate

2. MATERIALS AND METHODS

2.1. *Cell culture*

F9 teratocarcinoma cells were obtained from Dr Dorothe Spillman (Biocenter, University of Basel). The cell line was cultivated in Dulbecco's modified Eagle's medium supplemented with 10% fetal calf serum, 2 mM glutamine, 100 U/ml penicillin and 0.1 mg/ml of streptomycin (Gibco, Scotland) in a humidified 90% air/10% CO_2 incubator. The cells were induced by treating the cultures first with 0.5×10^{-6} M retinoic acid (Sigma) for 72 h, followed by a wash and then with 1×10^{-3} M dibutyryl-cAMP (Sigma) for 48 h according to [7] and collected. The morphology of the cells became clearly more rounded after 24 h in the presence of dibutyryl-cAMP. Untreated cells were collected from cultures grown for 5 days to confluency.

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2.2. *Extraction of HMG proteins*

HMG proteins and histone H1 were extracted from whole cells with 5% perchloric acid (PCA) at 0°C [9]. Proteins were precipitated by adding 100% trichloroacetic acid to a final concentration of 25% (w/v) and incubated at 0°C for 2 h before centrifuging for 40 min at 10000 × *g*. The precipitate was washed once with acetone/conc. HCl (400:1), once with acetone/0.1 M HCl (6:1), twice with acetone, and lyophilized.

2.3. *Polyacrylamide gel electrophoresis*

Electrophoresis was performed according to Panyim and Chalkley [10] using 15% polyacrylamide slab gels in acetic acid/2.5 M urea. Proteins treated with alkaline phosphatase [6] were analyzed using a discontinuous system described by Spiker [11]. Coomassie-stained proteins were analyzed by densitometry as in [12].

2.4. *Reversed-phase HPLC*

A Hewlett-Packard 1090 liquid chromatograph was used. The low-M_r HMG proteins were resolved on a Bio-Rad RP-304 (C4) column (250 × 4.6 mm) and the proteolytic fragments of HMG I on a Bio-Rad RP-318 (C18) column (250 × 4.6 mm) using a gradient of water/acetonitrile in 0.1% (v/v) trifluoroacetic acid. The eluted proteins were detected by their UV absorption at 214 nm and the peaks quantified on the basis of their areas using a Hitachi D-2000 integrator.

2.5. *Amino acid analysis and sequence determination*

The HPLC-purified proteins were hydrolyzed in 6 M HCl for 24 h and amino acids were analyzed as in [13]. Purified HMG proteins or proteolytic fragments of HMG I were sequenced using an Applied Biosystems 477A sequencer connected with 120 A analyzer. HMG I (8 μg) was digested for 2 h at 37°C with thermolysin (0.2 μg) in 20 μl of 0.1 M ammonium bicarbonate.

3. RESULTS AND DISCUSSION

Fig.1. shows the electrophoretic patterns of PCA- soluble proteins from confluent untreated F9 cells (control cells) and F9 cells, which were induced to differentiate by treatment with reinoic acid and dibutyryl-cAMP for 5 days (induced cells). Both control and induced cells contained the same major PCA-soluble proteins. However, densitometric analysis revealed that the levels of HMG I and HMG Y were markedly decreased and that of histone H1° increased in the induced cells. It has been previously reported [14] that levels of HMG 1 and HMG 2 decrease in mouse neuroblastoma cells induced to differentiate. We observed only a relatively small (26 ± 4%, n = 3) decrease in the content of HMG 2 and no appreciable change in HMG 1 in induced F9 cells. The total amount of H1 did not change significantly. This is in agreement with the results of Tan et al. [15]. However,

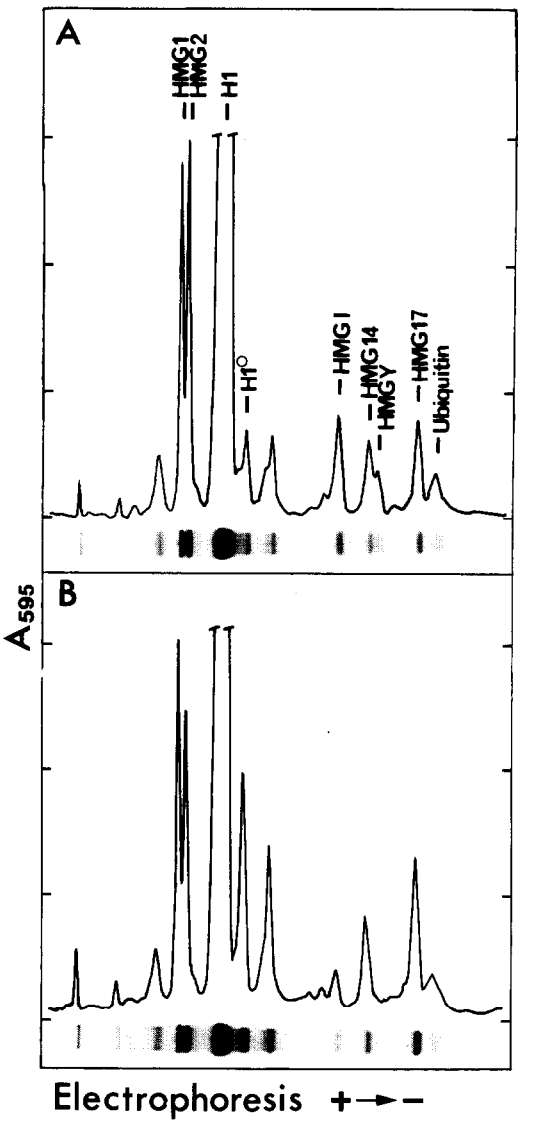

Fig.1. PCA-soluble proteins from untreated mouse teratocarcinoma F9 cells (A) and cells treated with retinoic acid and dibutyryl-cAMP (B) analyzed by acetic acid/urea-polyacrylamide gel electrophoresis and densitometry of Coomassie-stained gel. Proteins corresponding to 5 × 10^6 cells were loaded into both lanes. HMG proteins and ubiquitin from calf thymus were run in separate lanes as standards.

there was a 2.4 ± 0.4-fold (n = 3) increase in the relative proportion of histone H1° out of total histone H1.

In order to resolve and purify the low-M_r HMG proteins more efficiently, the proteins were separated using reversed-phase HPLC (fig.2).

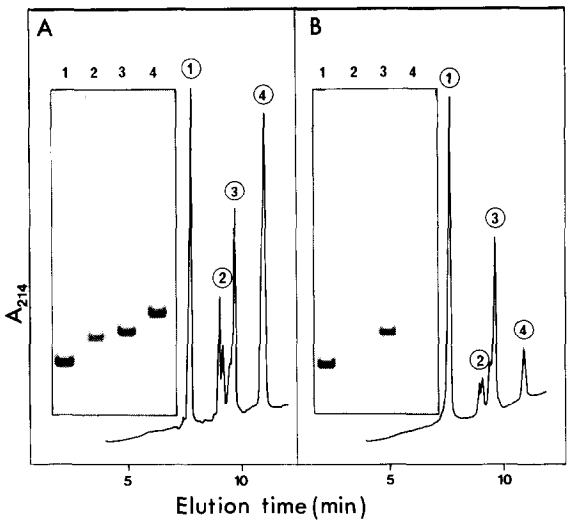

Fig.2. Comparison of elution profiles of low-M_r HMG proteins from untreated F9 cells (A) and cells treated with retinoic acid and dibutyryl-cAMP (B) chromatographed on a Bio-Rad C4 column. The protein peaks collected were analyzed by acetic acid/urea slab gel electrophoresis (shown as insets): HMG 17 (1), HMG Y (2), HMG 14 (3) and HMG I (4).

HMG 17, HMG 14 and HMG I eluted from the column as sharp peaks, whereas HMG Y was resolved into two peaks. The microheterogeneity of HMG Y was also detected in the Spiker gel electrophoretic system [11] and was abolished after alkaline phosphatase treatment in HMG Y (not shown) indicating that the peaks most probably represent differentially phosphorylated variants of the same protein. HMG Y has been shown to be phosphorylated in vivo in Ehrlich ascites, HeLa and CHO cells and can be phosphorylated in vitro by nuclear kinase II [6,13].

Using the HPLC method, it was estimated that the induced F9 cells contained 77 ± 6% ($n = 3$) less HMG I and 62 ± 2% ($n = 3$) less HMG Y than untreated F9 cells. At the same time, we did not observe any significant changes in levels of HMG 14 and HMG 17. Comparison of the amounts of HMG I and HMG Y in log vs plateau phase control cells did not reveal any significant changes (not shown).

The amino acid compositions of HMG I and HMG Y from F9 cells were found to be very similar (both containing less Asx and Ala and more Glx than HMG 14 and HMG 17) and comparable to those described by Lund et al. [6] for Ehrlich ascites cells. The final identification of the low-M_r HMG proteins (except HMG Y) from F9 cells was based on their partial sequencing. The N-terminal amino acid sequences of HMG 14 and HMG 17 were almost identical to those of calf thymus HMG proteins [16,17] (fig.3). Automated Edman degradation of HMG I and HMG Y did not yield amino acids, indicating that the N-terminal amino acids of both proteins were blocked.

To compare the amino acid sequence of HMG I from F9 cells with the partial sequence (105 amino acids) of HMG I from human placenta [18], HMG I from F9 cells was digested with thermolysin and the sequences of three HPLC-purified internal fragments (Th_A, Th_B, Th_C) were determined. The sequences of these peptides (a total of 50 amino acids) differed from that of the human protein only to a small extent, indicating that HMG I from

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                1                   10                  20
HMG 17          Pro-Lys-Arg-Lys-Ala-Glu-Gly-Asp-Ala-Lys-Gly-Asp-Lys-Thr-Lys-Val-Lys-Asp-Glu-Pro
HMG 17CT        I----------------------------------------------------Ala----------------------------

                1                   10
HMG 14          Pro-Lys-Arg-Lys-Val-Ser( - )Ala-Asp-Gly-Ala-Ala-Lys-Ala-Glu-Pro-Lys-Arg-Arg
HMG 14CT        I------------------------- Ser-----Glu---------------------- Glu----------------------
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Fig.3. N-terminal amino acid sequences of HMG 14 and HMG 17 from mouse F9 cells and the corresponding calf thymus (CT) proteins. Amino acid residues differing between the homologous proteins are indicated.

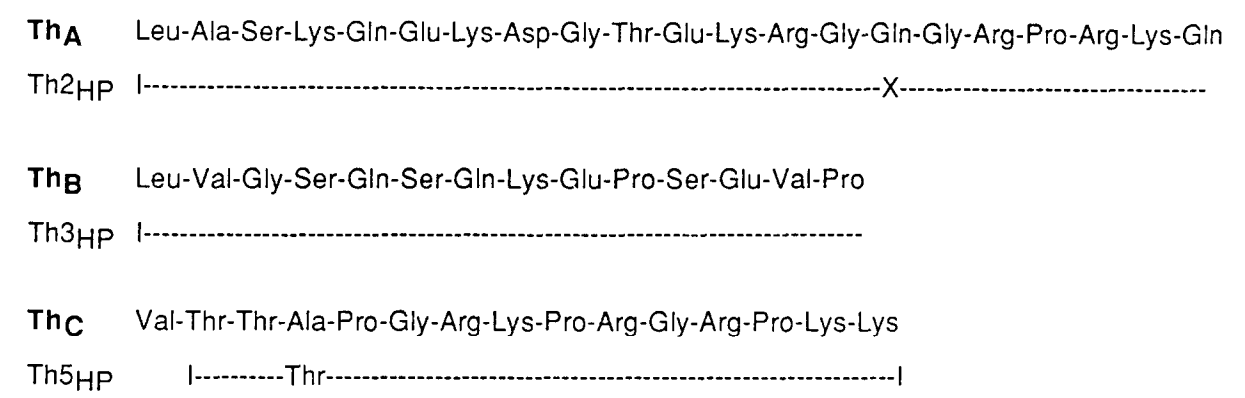

Fig.4. Amino acid sequences of the three internal thermolysin fragments of HMG I from F9 cells. Amino acid residues which are different from those of HMG I from human placenta are indicated. (X: unidentified residue).

F9 cells is highly homologous with HMG I from human cells (fig.4). The unidentified residue (X) in the human protein was identified as Gln in the mouse protein. Furthermore, the N-terminal end of the Th_C-peptide from F9 cells (Val-Thr-Thr-Ala) differed from that in the corresponding human protein (Thr-Thr-Thr-Thr) [18]. The palindromic amino acid sequences Lys-Pro-Arg-Gly-Arg-Pro-Lys, which may play a role in binding of HMG I to A-T-rich sequences in the minor groove of DNA [18,19], is also found in mouse HMG I.

The significant decrease in relative amount of HMG I during teratocarcinoma cell differentiation is probably related to the disappearance of a previously unidentified low-M_r HMG protein, HMG 9, in F9 cells treated with retinoic acid [15]. On the basis of the present results, we suggest that HMG 9 and HMG I are identical. HMG I has been demonstrated in proliferating human, monkey, mouse and rat cells [6,19,20] but not in transformed chicken erythrocytes, which had progressed to a late stage along the differentiation pathway [20]. HMG Y has been found in human, mouse and hamster cells [6,13]. The present results suggest that HMG I and HMG Y, both phosphoproteins, are HMG proteins specific for undifferentiated cells. Further binding and cross-linking studies are, however, required to elucidate and confirm the involvement of these proteins in maintaining the undifferentiated state of chromatin.

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