HMG 17 IN METAPHASE-ARRESTED AND INTERPHASE HeLa S3 CELLS

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1. Introduction

Proteins HMG 17 and 14 belong to a group of proteins designated high mobility group proteins [1]. Experiments have indicated that transcribed genes are enriched in HMG 17 and 14 or the homologous protein H6 [2–5]. The exact location of these proteins on the nucleosomes of transcribed sequences has not yet been settled. Certain experiments indicate that in the transcribed regions HMG 17 and 14 replace H1, and it has been suggested that this results in a local unfolding of chromatin, thereby facilitating transcription [6]. On the other hand, these proteins may not only play a role during transcription since they remain attached to extensively digested nucleosomes [6] and reconstitution experiments [7,8] indicate that HMG 17 and 14 readily bind to the 145 base pair core particle at a stoichiometry of 1 and 2 molecules/core.

During mitosis chromatin is condensed into mitotic chromosomes with a factor of condensation of ~250. If the presence of HMG 17 and 14 results in an unfolding of certain regions of the genome one can ask if these proteins are present in metaphase chromosomes. Furthermore, it would be interesting to know whether or not these proteins are present in modified form during mitosis.

Here, we have compared HMG 17 isolated from HeLa S3 interphase and metaphase-arrested cells. In addition, metaphase chromosome clusters have been examined for the presence of HMG 17. The results indicate that, compared to interphase cells, metaphase-arrested cells contain modified forms of HMG 17. Furthermore, isolated chromosome clusters were found to contain HMG 17.

Abbreviations: PMSF, phenylmethyl sulfonyl fluoride; PCMPS, *p*-chloromercuriphenyl sulfonate

2. Materials and methods

2.1. *Propagation of HeLa S3 cells*

Interphase cells were grown in suspension culture as in [9].

To obtain cells arrested in metaphase, cells were grown in roller tubes and harvested after treatment with colcemid (0.05 μg/ml) as in [9]; 95% of the cells were in metaphase.

2.2. *Isolation of metaphase chromosome clusters*

These were prepared by a method developed in [10,11] and include a Percoll gradient centrifugation. All steps were carried out at 0°C.

The Percoll gradient was made by diluting a Percoll stock solution (Pharmacia) until final concentration: 60% Percoll, 10 mM Hepes (pH 7.2), 10 mM NaCl, 10 mM $MgCl_2$, 0.5 M sucrose, 0.5 mM $CaCl_2$ and 0.1% Nonidet P 40 (NP 40). The Percoll solution was then centrifuged at 48 000 × *g* for 40 min in an angle head rotor.

Harvested metaphase cells were washed once in ice-cold 0.9% NaCl and pelleted cells resuspended (10^7 cells/ml) in a buffer containing 10 mM Hepes (pH 7.2), 10 mM NaCl, 10 mM $MgCl_2$, 0.5 mM $CaCl_2$, 0.5 M sucrose, 0.5 mM PMSF, 2 mM PCMPS and 0.1% NP 40. The suspension was subjected to 20 strokes in a tight-fitting Dounce homogenizer to obtain maximum number of mitotic nuclei. To remove the main bulk of cytoplasmic material the homogenate was centrifuged at 2000 × *g* for 10 min and the pellet resuspended in the same volume of buffer. Suspension (3 ml) was layered over 10 ml Percoll gradient in a 15 ml Corex tube and centrifuged at 2500 × *g* for 1 h (swinging bucket rotor). The purified chromosome clusters collected ~1 cm from the bottom of the tube were diluted with a buffer consisting of

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20 mM Tris–HCl (pH 7.3), 1 mM $CaCl_2$, 1 mM $MgCl_2$ and 1 mM $ZnCl_2$, centrifuged and the pellet washed twice with the same buffer.

2.3. *Preparation of protein HMG 17*

Interphase and metaphase-arrested HeLa S3 cells and metaphase chromosome clusters were extracted with perchloric acid [1] at 0°C. To 10^8 cells (or metaphase chromosome clusters corresponding to 10^8 cells) was added 1 ml 1.5 M perchloric acid followed by 2 ml 0.75 M perchloric acid, homogenized in a Dounce homogenizer, left for 1 h and centrifuged. The pellet was re-extracted for 1 h with 1.5 ml 0.75 M perchloric acid and centrifuged. The combined supernatants was added HCl (final conc. 0.07 N), 6 vol. acetone and left for 18 h at –20°C. After centrifugation the pellet was washed in acetone–HCl and finally in acetone.

HMG 17 was purified by electrophoresis in a slab gel containing 15% acrylamide, 0.05% bisacrylamide, 2.5 M urea and 0.9 N acetic acid [12]. The acetone precipitate dissolved in acetic acid–urea was applied across a 15 cm wide slab gel, 3 mm thick, and run for 3 h at 200 V. The gel was stained for 10 s in 0.1% Coomassie brilliant blue R. The band corresponding to the position of HMG 17 was cut out and the protein eluted from the gel electrophoretically into 0.9 N acetic acid. The eluted proteins were precipitated with trichloroacetic acid (final conc. 25%) and was washed twice with acetone. The yield of HMG 17 from 10^8 cells was ~5 μg.

For comparative purposes HMG 17 was extracted from pig thymus and purified by the electrophoretic procedure above.

2.4. *Polyacrylamide gel electrophoresis of proteins*

Acetic acid–urea gel (15% acrylamide, 0.05% bisacrylamide and 2.5 M urea and 0.9 N acetic acid) was done as in [12]. In some cases stacking gel containing 7.5% acrylamide, 0.05% bisacrylamide, 0.375 M potassium acetate buffer (pH 4.0) and 2.5 M urea was used [13]. Triton X-100–acetic acid–urea gel was as in [14]. SDS gel electrophoresis was as in [15]. In all cases gels were stained in 0.1% Coomassie brilliant blue R.

Gels were scanned at 600 nm in a Gilford spectrophotometer.

2.5. *Pepsin digestion of HMG 17*

The protein (10 μg) was dissolved in 5% acetic acid (10 μl) and incubated for 12 h at 37°C with pepsin (0.1 μg). The lyophilized sample was submitted to gel electrophoresis [16].

2.6. *Amino acid analysis*

This was done with 10 μg protein at the Central Amino Acid Analysis Laboratory, Institute of Biochemistry, Uppsala.

3. Results and discussion

Whole cells arrested in metaphase and logarithmically growing cells were extracted with perchloric acid and the HMG 17 protein purified by preparative gel electrophoresis (section 2). The purity of the products was then examined. In the Triton X-100–acetic acid–urea system with stacking gel (fig.1), HMG 17 from metaphase-arrested cells (lanes a,b)

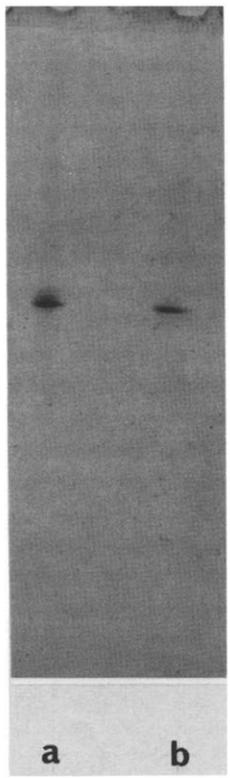

Fig.1. Polyacrylamide (15%) Triton X-100–acetic acid–urea gel electrophoresis (with stacking gel): (a) purified HMG 17 from metaphase cells; (b) purified HMG 17 from interphase cells.

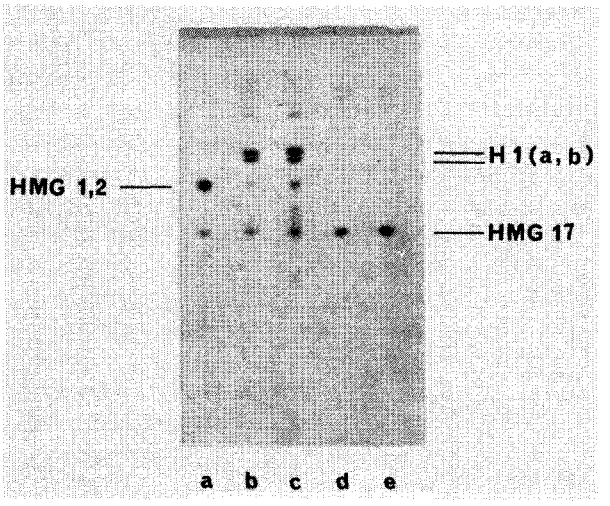

Fig.2. Polyacrylamide (15%)–SDS gel electrophoresis: (a) high mobility group fraction from pig thymus; (b,c) acetone precipitate of perchloric acid extracts from interphase and metaphase-arrested cells, respectively; (d,e) purified HMG 17 from metaphase and interphase cells, respectively.

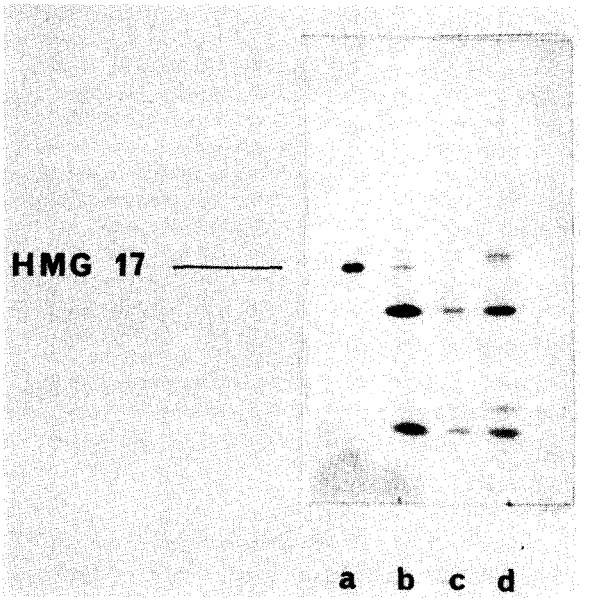

Fig.3. Polyacrylamide (15%) Triton X-100–acetic acid–urea gel electrophoresis (with stacking gel) of pepsin digestion of HMG 17 proteins: (a) HMG 17 from pig thymus; (b–d) pepsin digestion of HMG 17 from pig thymus, interphase and metaphase-arrested cells, respectively.

gave one main band with two marginally slower moving bands (see also scan in fig.4). HMG 17 from interphase cells, however, gave only one band with identical mobility to the main band from metaphase cells. In the SDS gel system (fig.2), HMG 17 from the two types of cells gave only one band with identical mobility. Hence it seems likely that the heterogeneity of HMG 17 from metaphase cells may be due to chemical modification.

To obtain further proof for the presence of modified HMG 17 proteins, pepsin digestion which is known to split HMG 17 at the leucine residue 27 [16] in two fragments was attempted. The fragments obtained were separated by gel electrophoresis. The faint bands (fig.3) found in the position of HMG 17 was due to incomplete pepsin digestion. HMG 17 from interphase cells gave two fragments (fig.3, lane c) corresponding to the mobility of those obtained with HMG 17 from pig thymus (lane b). HMG 17 from metaphase-arrested cells (lane d) gave two bands with mobility equal to those obtained from interphase cells but an additional band was present. When the gel was run in SDS in a second dimension the additional fragment exhibited the same mobility as the fragment with the highest mobility in the first dimension, indicating similar molecular size (~3000 dalton) for the two fragments (not shown). It seems likely that the additional fragment obtained after pepsin digestion has arisen from the protein corresponding to peak 2 in fig.4 and that the modification is located in the fragment containing the N-terminal end of HMG 17. The lower mobility could be the result of phosphorylation, ADP-ribosylation or acetylation. Other workers have reported phosphorylation of HMG 17 in Ehrlich ascites cells [17] and in HeLa cells after sodium butyrate treatment [18].

To get an estimate of the ratio between the 3 bands in the HMG 17 preparation from metaphase cells a Triton X-100–acetic acid–urea gel was scanned. The scan is seen in fig.4 and gives a ratio of 12:3:1.

By scanning acetic acid–urea gels of acetone precipitates of perchloric acid extracts of the cells, the ratio between H1 and HMG 17 in metaphase and interphase cells were found to be about the same.

HMG 17 had not been isolated from HeLa S3 cells. To characterize the protein an amino acid analysis was carried out and the results compared with the amino acid composition of HMG 17 isolated by an identical method from pig thymus. It is seen from table 1 that the amino acid composition of the two proteins is very similar.

Clusters of metaphase chromosomes were then examined for the presence of HMG 17. To prevent

degradation of proteins the metaphase clusters were isolated in the presence of PMSF and PCMPS. The method used yielded a product microscopically free from cytoplasma (protein/DNA, 1.5). It is seen from fig.5 (lane a) that a perchloric extract of metaphase

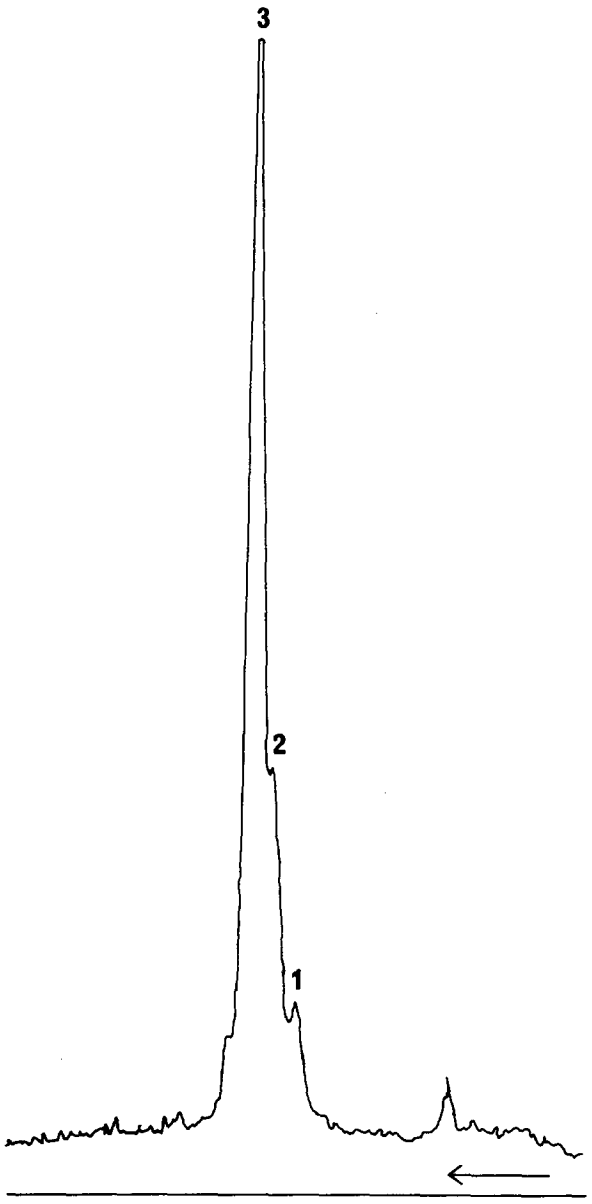

Fig.4. Scan of stained 15% polyacrylamide Triton X-100–acetic acid–urea gel (with stacking gel) of HMG 17 from metaphase-arrested cells. Arrow indicates direction of movement. Peak 3 corresponds to the band with equal mobility to that of HMG 17 from interphase cells. Peak 1 and 2 indicate the position of the modified proteins.

Table 1
The amino acid composition (% mol) of HMG 17 protein from HeLa S3 cells (interphase) and pig thymus

	HeLa S3 cells	Pig thymus
Asp	10.5	11.2
Thr	2.4	2.3
Ser	4.5	4.3
Glu	11.5	10.5
Pro	10.8	11.1
Gly	11.8	11.2
Ala	15.4	16.4
Val	2.6	2.8
Met	Trace	–
Ile	1.1	0.5
Leu	2.4	2.0
Tyr	Trace	0.6
Phe	Trace	Trace
His	Trace	Trace
Lys	20.7	21.4
Arg	4.6	4.9

No corrections have been made for hydrolytic losses

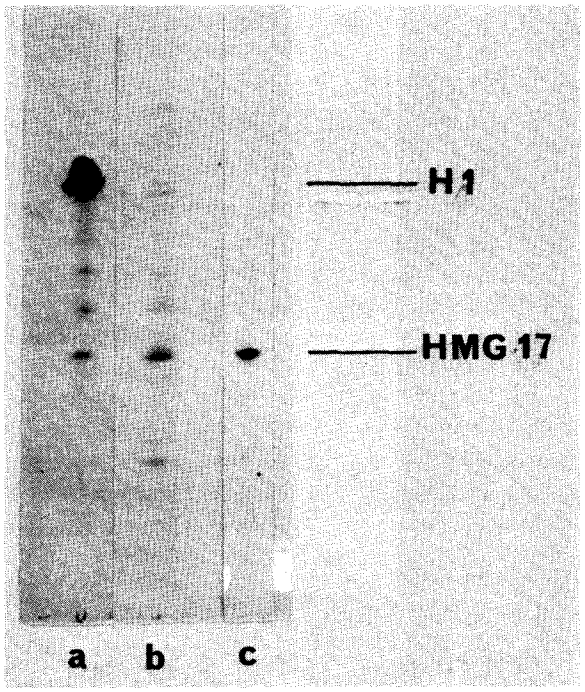

Fig.5. Polyacrylamide (15%) acetic acid–urea gel electrophoresis: (a) acetone precipitate of perchloric extract of metaphase chromosome clusters; (b) acetone precipitate of perchloric acid extract of cytoplasmic proteins from metaphase-arrested cells; (c) HMG 17 from interphase cells.

chromosomal clusters does contain HMG 17. When isolating these chromosomes, cells are treated with the ISB buffer (see methods) containing the detergent NP-40. The cytoplasmic fraction obtained (section 2) was also examined for the presence of HMG 17. It is seen that (fig.5, lane b) the cytoplasmic fraction also contains HMG 17. An approximate estimate is that ~30% of the total HMG 17 is in the cytoplasmic fraction. Whether or not HMG 17 is present in the cytoplasmic fraction of metaphase-arrested cells is difficult to decide since it may well be that the isolation procedure strips of some HMG 17 from the metaphase chromosomes.

The finding that HMG 17 is present in metaphase chromosomes where RNA synthesis has ceased [19] is compatible with the view that this protein is not only associated with transcribed sequences as, e.g., by its presence in satellite chromatin [20]. Furthermore it is interesting that in the metaphase-arrested cells part of HMG 17 seems to be modified when compared with interphase cells. The modification could well play a role in the condensation of chromatin during mitosis.

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