On the presence of two new high mobility group-like proteins in HeLa S3 cells

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Two phosphorylated HMG-like proteins with M_r ~10 000 have been isolated from HeLa S3 cells, one being present in metaphase and one in interphase cells. The amino acid compositions of these proteins are very similar but differ from the known HMG proteins. However, they exhibit similarities being rich in proline, basic and acidic amino acids. A possible role in chromatin condensation of the HMG-like protein characteristic for metaphase cells is suggested.

High mobility group-like protein *Interphase* *Metaphase* *Phosphorylation*

1. INTRODUCTION

Postsynthetic modifications of chromatin-associated proteins are a means to alter chromatin structure. Investigations of such modifications are of importance for the understanding of gene expression and chromatin condensation during mitosis. Of the non-histone proteins, the high mobility group (HMG) proteins which can be extracted with 5% perchloric acid [1] may be modified by acetylation [2], methylation [3], ADP-ribosylation [4] and phosphorylation [5–9]. Although evidence indicates that HMG 14 and 17 are associated with transcribed sequences [10–12], the biological function of the HMG proteins, in particular their modified forms, is poorly understood.

Continuing our previous study [13] on the modification of HMG proteins in HeLa S3 metaphase and interphase cells, we have investigated the incorporation of [^{32}P]phosphate into 5% perchloric acid extracts of such cells. These studies have led to the detection of phosphorylated HMG-like proteins which do not seem to have been described before. Here, we report on the isolation and amino acid composition of two of these proteins. One is present in metaphase and the other in interphase cells. Furthermore we show that HMG 14 and 17 are phosphorylated in metaphase cells whereas in contrast to others [5–9] we do not find phosphorylation of these proteins in interphase cells.

Abbreviations: PMSF, phenylmethyl sulphonyl fluoride

2. MATERIALS AND METHODS

2.1. *Propagation of HeLa S3 cells*

To obtain cells arrested in metaphase, these were grown in roller tubes and harvested after treatment with colcemid (0.15 μg/ml) as in [14]. Interphase cells were grown in suspension culture as in [14].

2.2. *Labelling of metaphase and interphase cells with [^{32}P]phosphate*

Carrier free phosphate (0.03 mCi/ml) and thymidine (2.5 mM) were added to the medium 4 h before harvesting metaphase arrested cells. 85% of the cells obtained were in metaphase.

Suspension cultures of interphase cells were centrifuged and the cells suspended in fresh medium (128×10^4 cells/ml) containing carrier free [^{32}P]phosphate (0.03 mCi/ml) and harvested after 4 h incubation at 37°C.

2.3. *Extraction of cells with perchloric acid*

Cells washed with cold saline containing 0.5 mM PMSF were extracted with perchloric acid at 0°C as in [13].

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2.4. *Polyacrylamide gel electrophoresis of proteins*

Acetic acid–urea gel electrophoresis (15% acrylamide, 0.5% bisacrylamide, 2.5 M urea and 0.9% acetic acid) was done as in [15]. In all cases stacking gel containing 7.5% acrylamide, 0.05% bisacrylamide, 0.0375 M potassium acetate buffer (pH 4.0) and 2.5 M urea was used [16]. SDS–gel electrophoresis was as in [17]. All gels were stained with 0.1% Coomassie brilliant blue R. For autoradiography dried gels were exposed to Kodak X-omatic RP film.

For preparative purposes acetic acid–urea (as above) slab gel (15 cm wide and 1.5 mm thick) was used. Proteins from 5×10^8–10^9 cells were applied on one gel. The gel was stained for 10 s in 0.1% Coomassie brilliant blue G. Proteins were eluted from gel strips electrophoretically in 0.9 N acetic acid and collected against a Nucleopore UF ultrafilter type C with M_r 5000 cut-off, precipitated with trichloroacetic acid (25%), and washed with acetone.

2.5. *Phosphatase treatment of ^{32}P-labelled proteins*

Proteins from 10^7 cells were incubated at 37°C for 16 h with a mixture containing 100 mM Tris–HCl (pH 8.0), 0.1 mM $ZnCl_2$, 1 mM $MgCl_2$, 1 mM PMSF and 10 μg *Escherichia coli* phosphatase (Sigma Type III R) in a total volume of 20 μl. Protein was precipitated with trichloroacetic acid and washed with acetone.

3. RESULTS

HMG proteins were extracted from whole cells with 5% perchloric acid and the soluble proteins from metaphase and interphase cells were first

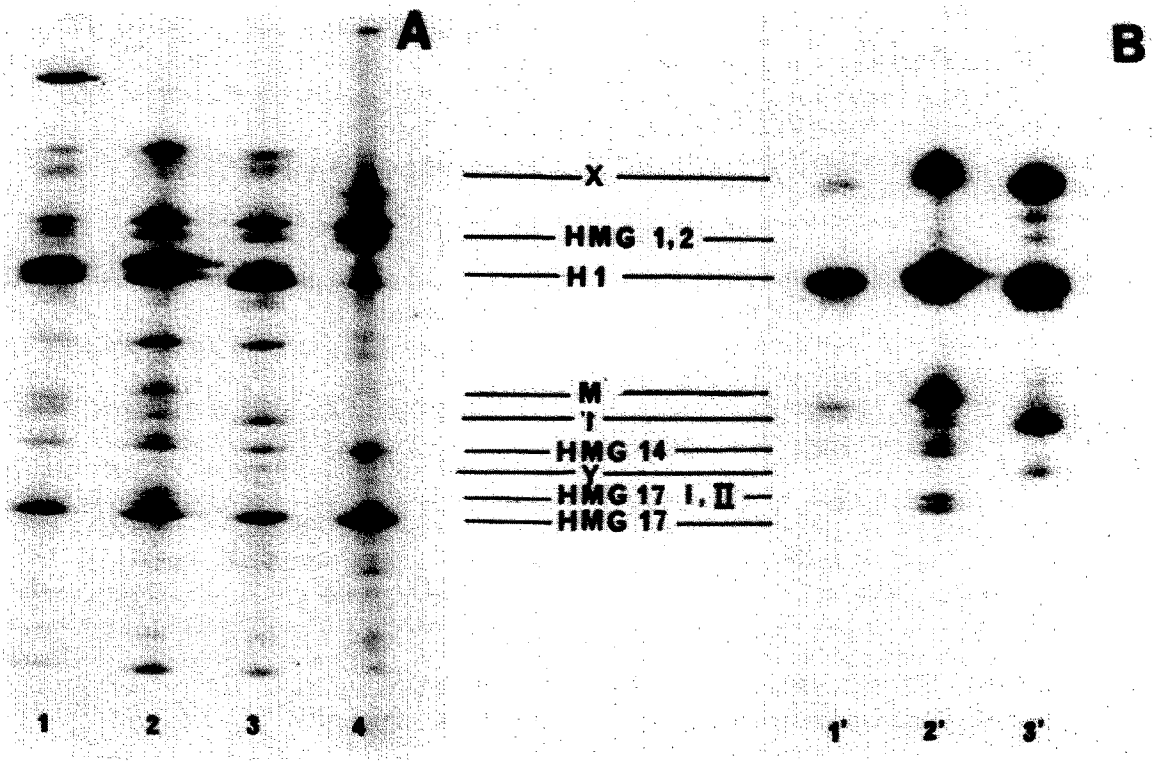

Fig.1. Acetic acid–urea polyacrylamide gel electrophoresis of 5% perchloric acid extracts of metaphase and interphase cells labelled with [^{32}P]phosphate. Material corresponding to 10^7 cells was applied on the gel. (A) Coomassie stained gel; (B) autoradiograph of gel. Lanes 1 and 1′, phosphatase treatment of perchloric acid extracted proteins from metaphase cells; lanes 2 and 2′ from metaphase cells; lanes 3 and 3′ from interphase cells; lane 4, HMG proteins from thymus.

analysed by electrophoresis in acetic acid–urea gels (fig.1A, lane 2 and 3). H1, HMG 1, 2, 14 and 17 are seen together with some other proteins. The autoradiograph of the gel is seen in fig.1B. Both metaphase and interphase cells contain a heavily labelled unidentified band designated X and labelled H1. As expected, H1 in metaphase exhibits a lower mobility due to superphosphorylation [18]. Furthermore, it is seen that metaphase cells contain a labelled protein designated M (fig.1B, lane 2′) which is barely visible in interphase cells (fig.1B, lane 3′). On the other hand, interphase cells contained a labelled protein designated I (fig.1B, lane 3′) only just visible in the metaphase preparation which contained 15% of cells in interphase (see section 2). In two-dimensional electrophoresis, the M and I protein gave each rise to only one labelled Coomassie staining spot (fig.2A and B and 3A and B). In the SDS dimension the M protein had about the same mobility as HMG 14 and the I protein a mobility marginally slower than HMG 17.

To isolate sufficient quantities of the M and I protein for amino acid analysis, preparative acetic acid–urea gel electrophoresis was carried out. The yield of M and I protein was 2 μg and 2.5 μg per 10^8 cells, respectively. The isolated proteins gave only one Coomassie stained band upon analysis in acetic acid–urea or SDS gels. The amino acid compositions of these two proteins together with those of HMG 14 from thymus and HMG 17 from HeLa interphase cells are given in table 1. It is seen that the amino acid compositions of the M and I protein are quite similar. Compared to HMG 14 and 17, there are some striking differences. The M and I protein contains considerably more serine and less alanine. They are similar to the HMG proteins

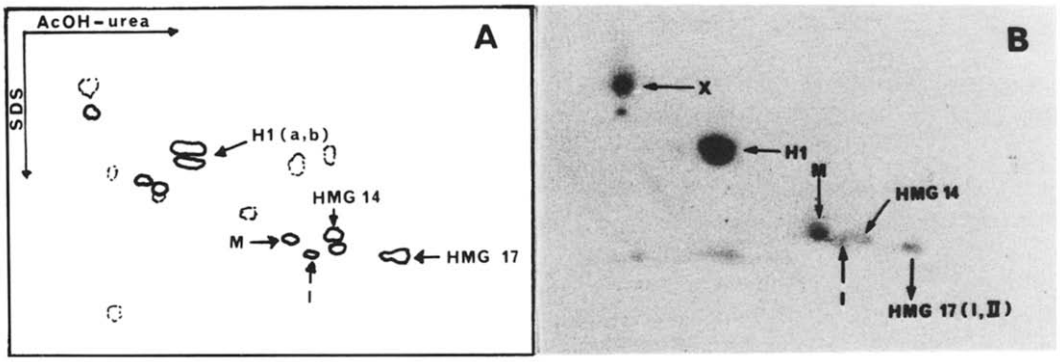

Fig.2. Two-dimensional polyacrylamide gel electrophoresis of 5% perchloric acid extract from metaphase cells labelled with [^{32}P]phosphate. (A) Coomassie stained gel; (B) autoradiograph of gel.

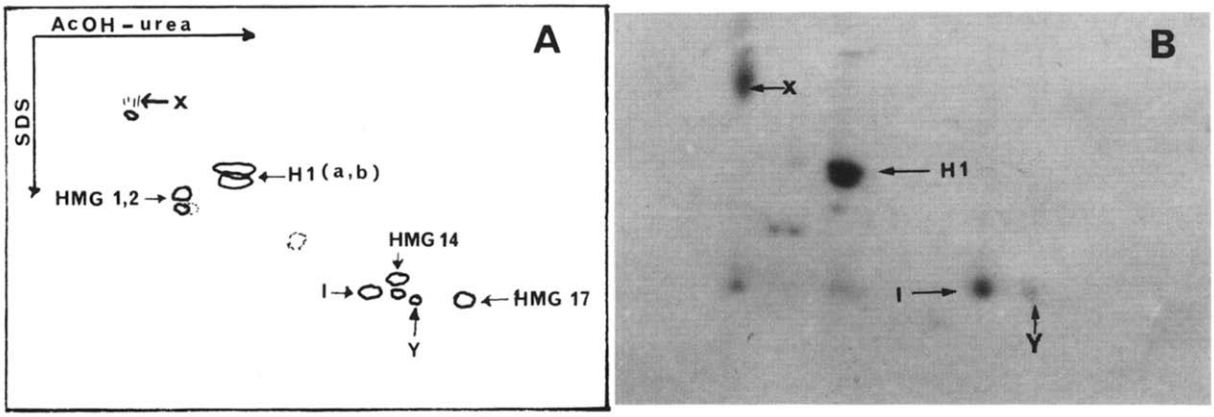

Fig.3. Two-dimensional polyacrylamide gel electrophoresis of 5% perchloric acid extract of interphase cells labelled with [^{32}P]phosphate. (A) Coomassie stained gel; (B) autoradiograph of gel.

Table 1

Amino acid composition (mol %)

	HMG 17[a] (HeLa)	HMG 14[b] (Thymus)	HMG M	HMG I
Asp	10.5	8.3	3.3	3.0
Thr	2.4	4.1	6.2	7.2
Ser	4.5	8.0	12.2	12.2
Glu	11.5	17.5	18.4	18.6
Pro	10.8	8.1	10.2	10.5
Gly	11.8	6.4	10.8	10.4
Ala	15.4	14.8	5.8	5.3
Val	2.6	4.0	3.0	3.2
Met	Trace	0.1	–	–
Ile	1.1	0.3	1.5	1.2
Leu	2.4	2.0	2.9	3.3
Tyr	Trace	0.2	–	–
Phe	Trace	0.3	–	–
Lys	20.7	21.1	15.0	15.0
His	Trace	0.2	1.1	0.4
Arg	4.6	5.4	8.5	9.0

[a] from [13]
[b] from [19]

in being rich in acidic and basic amino acids. Provided the M and I proteins behave in SDS gel electrophoresis similar to that of HMG 14 and 17, the M protein would have a M_r about the same as HMG 14 (10000) and the I protein in between HMG 14 and 17 (about 9500).

In addition to the M protein the autoradiograph of the acetic acid–urea gel from metaphase cells (fig.1B, lane 2′) reveals the presence of a labelled band corresponding to the position of HMG 14 and two labelled bands with mobility slower than HMG 17. These latter two bands (HMG 17 I and II) correspond to the two modified HMG 17 proteins described by us in [13]. Phosphatase treatment of the perchloric acid soluble proteins from metaphase cells removed the label in the position of HMG 14 and the two modified HMG 17 proteins (fig.1B, lane 1′). It is seen that interphase cells do not contain labelled HMG 14 and 17 (fig.1B, lane 3′). On the other hand, interphase cells contained an unidentified band designated Y which is absent in metaphase cells. In the acetic acid–urea gel it had a mobility higher than HMG 14 and in the SDS dimension the mobility was equal to that of HMG 17 (fig.3A and B).

4. DISCUSSION

We have considered the possibility that the M and I proteins could be proteolytic degradation products. As cells were extracted with perchloric acid immediately after harvesting and their amino acid compositions seem to exclude the possibility that they were derived from H1 or HMG 1 and 2 by proteolysis, we do not consider this a very likely possibility. The amino acid composition of the M and I protein is interesting in that they are rich in serine, thus they have the structural capacity for extensive phosphorylation which may be of importance for their biological function. Phosphatase treatment of perchloric acid extracts from metaphase cells (fig.1B, lane 1′) and interphase cells (not shown) demonstrated that the M and I protein were phosphorylated.

Previous work has shown that the M protein was present in mitotic nuclei [13] and hence seems to be a constituent of the mitotic apparatus. Since it does not seem to be present in interphase cells, it may be of importance in the mitotic process. It has been reported [20] that metaphase chromosomes from HeLa cells contain mitotic factors which induce chromosome condensation when injected into fully grown *Xenopus laevis* oocytes and induce meiotic maturation. The activity is soluble in 2% trichloroacetic acid, extractable with 0.2 M NaCl and thus resemble HMG proteins. It is tempting to speculate that the M protein could be one of these mitotic factors.

The I protein was localized in the nucleus since it could be extracted from nuclei isolated from interphase cells (not shown). Whether this interphase protein is present or phosphorylated to the same extent in G_1, S, G_2 remains to be seen.

The M and I proteins are soluble in 5% perchloric acid, have high mobility in the acetic acid–urea gel system and are rich in acidic and basic amino acids. Tentatively we would like to suggest the name HMG M and HMG I for these proteins. The striking similarity in the amino acid composition of the two proteins may suggest that they could represent the same polypeptide chain modified to different degrees.

Our results also confirm the presence in metaphase cells of phosphorylated HMG 14 [9]. However, in contrast [5–8] we do not find phosphorylation of HMG 14 and 17 in interphase cells. A possi-

ble reason for the conflicting results could be that the I protein and possibly the Y protein were mistaken for phosphorylated HMG 14 or 17. Our results show that in SDS–gel electrophoresis (fig.3A and B), the I protein has a mobility only marginally slower than HMG 17 and the mobility of the Y protein is very similar to HMG 17. So, if protein was analysed only by SDS–gel electrophoresis [5,6], the phosphorylated I and Y proteins may be mistaken for phosphorylated HMG 17. In the acetic acid–urea gel, the I protein migrates marginally slower than HMG 14 and could therefore be mistaken for phosphorylated HMG 14 [7]. The large increase in the phosphorylation of HMG 14 in the G_2 phase in HeLa cells as reported in [6] could, if cells were examined late in the G_2 phase, have been caused by the appearance of the phosphorylated M protein.

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