HMG-like proteins of *Physarum polycephalum:* Association with the transcriptionally active chromatin

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HMG-like proteins were isolated from nuclei of the acellular slime mold *Physarum polycephalum*. These proteins represented 0.6 ± 0.2% of nuclear proteins and approx. 6% of DNA by weight. Polyacrylamide gel electrophoresis demonstrated the presence of 3 major proteins, designated HMG-1P, HMG-2P and HMG-14/17P. None of these proteins comigrated with any of the calf thymus HMGs. *Physarum* HMG-like proteins were found to be preferentially associated with transcriptionally active chromatin fraction, as well as being released from nuclei by light DNase I digestion. In contrast, the content of histone H1 was significantly lower in active than in inactive chromatin fraction.

Physarum polycephalum *High mobility group protein* *Transcriptionally active chromatin*

1. INTRODUCTION

The HMG proteins of animal cells are at present the most extensively characterized non-histone chromosomal proteins. In both mammals and birds 4 major proteins (HMG 1, HMG 2, HMG 14, HMG 17) occur, which are closely related in all species studied. Homologous proteins, named HMG-T1, HMG-T2 and H6, have been identified in fishes [1]. There is considerable evidence that HMG 14 and HMG 17 are involved in maintaining the structure of transcriptionally active chromatin [2].

HMG-like proteins have been also found in insects [3–5], *Tetrahymena* [6,7] and yeast [8,9], as well as in plants [8]. As compared to vertebrates, HMG-like proteins of lower eukaryotes are poorly characterized with respect to both molecular properties and a function in chromatin. Existing data clearly demonstrate, however, that these proteins differ significantly from vertebrate HMGs, at least in amino acid composition and electrophoretic behaviour.

We report here on the isolation of HMG-like proteins from the acellular slime mold *Physarum polycephalum*. We find that this primitive eukaryote contains 3 major proteins of the HMG, type, different from those of animal cells in electrophoretic behaviour, but similarly localized preferentially in the transcriptionally active chromatin.

Abbreviations: HMG, high mobility group; PMSF, phenylmethylsulphonyl fluoride

2. EXPERIMENTAL

2.1. Growth of microplasmodia of P. polycephalum

Microplasmodia (strain M_3CIV) were grown in submerged shaken cultures as in [10]. Plasmodia were harvested 48 h after inoculation.

2.2. Isolation of nuclei

Nuclei were isolated from freshly harvested or previously frozen microplasmodia as in [11]. All solutions contained 0.1 mM PMSF to inhibit proteolysis.

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2.3. Isolation of HMG-like proteins

Isolated nuclei were washed once in a solution containing 0.25 M sucrose, 10 mM NaCl, 3 mM $MgCl_2$, 10 mM Tris–HCl (pH 7.8) and then twice in 75 mM NaCl, 25 mM EDTA (pH 7.8). Purified nuclei were lysed in 1 mM Tris–HCl (pH 7.8) and centrifuged at 7000 × *g* for 15 min. HMG-like proteins were extracted from the resulting pellet with 0.35 M NaCl as in [12] and then recovered from the extract by the ammonium sulphate method of [13].

2.4. Chromatin fractionation

Transcriptionally active and inactive chromatin fractions were obtained by the modified method of [14], as described in [15].

2.5. DNase I digestion of nuclei

Nuclei were suspended in a buffer containing 0.25 M sucrose, 0.1 mM $MgCl_2$, 10 mM Tris–HCl (pH 7.8) and incubated with DNase I at 1×10^{-3} μg/ml, for 10 min at 37°C. Reaction was terminated by chilling and adding 5 mM EDTA. The digest was immediately centrifuged at 15000 × *g* for 15 min, and the supernatant used for analysis of solubilized proteins. The extent of DNA hydrolysis, expressed as the percentage of total nuclear DNA rendered soluble in 0.8 M perchloric acid, was determined in 200-μl aliquots removed from the digest.

2.6. Polyacrylamide gel electrophoresis of proteins

Electrophoresis was carried out in 15% polyacrylamide-SDS slab gels (17 × 17 × 0.2 cm) as in [16]. The gels were stained with 0.1% Coomassie brilliant blue [16] or silver [17]. Calf thymus HMG proteins isolated as in [12] were used as a standard. For the estimation of M_r values (in parenthesis) the following marker proteins were included in the gel: phosphorylase *b* (94000), bovine serum albumin (67000), ovalbumin (43000), carbonic anhydrase (30000), soybean trypsin inhibitor (20100) and α-lactalbumin (14400).

2.7. Determination of protein and DNA

Protein content was determined as in [18], and DNA content as in [19], using as standards bovine serum albumin and calf thymus DNA, respectively.

3. RESULTS

3.1. Isolation and electrophoretic characteristic

The HMG proteins are extracted from chromatin or nuclei using either 0.35 M NaCl or perchloric acid, and then recovered from the extract by fractional precipitation with trichloroacetic acid, acetone (see [1]) or ammonium sulphate [13]. When this procedure was applied to the nuclei of *P. polycephalum* we obtained a fraction of proteins, which followed the extractability properties of the HMG proteins. This fraction represented a relatively constant portion of total nuclear proteins, amounting 0.6 ± 0.2% (data from 5 experiments). The weight ratio of the HMG-like protein fraction to DNA was estimated to be approx. 0.06. We found, however, that the use of perchloric acid or trichloroacetic acid for preparation of *Physarum* HMG-like proteins led to irreversible aggregation

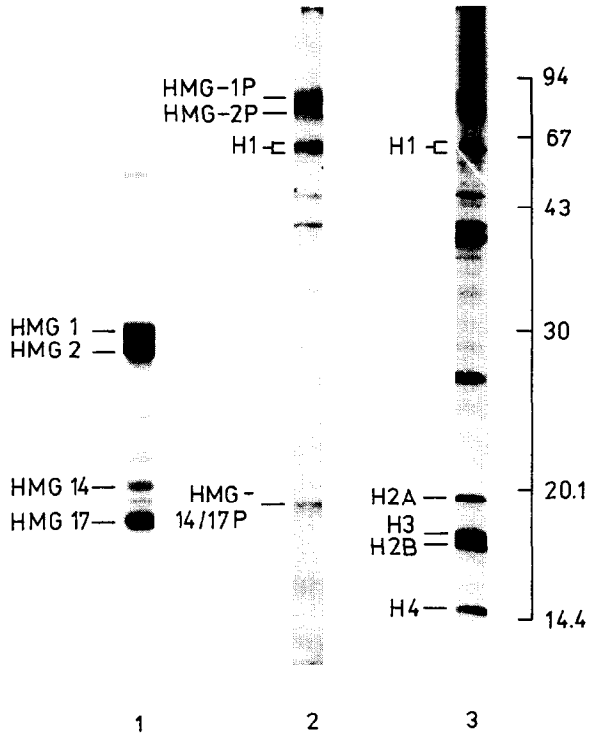

Fig.1. Electrophoretic pattern of *Physarum* HMG-like proteins. (1) Calf thymus HMG proteins, (2) HMG-like proteins of *Physarum*, (3) total *Physarum* nuclear proteins. The molecular mass values (in kDa) of marker proteins are shown on the right as size references. Coomassie staining.

of proteins. This could be avoided by extraction of nuclei with 0.35 M NaCl, and subsequent fractionation of extracted proteins with ammonium sulphate.

An SDS-gel electrophoretic analysis of the isolated protein fraction showed the presence of histone H1 and 3 major non-histone proteins, running at 82, 75 and 18.7 kDa (fig.1). None of the *Physarum* proteins comigrated with any of the calf thymus HMGs. However, their electrophoretic behaviour suggested the classification of the 82 and 75 kDa proteins among the 'large' HMGs, and that of the 18.7 kDa protein among the 'small' HMGs. By analogy to mammalian proteins, these proteins of *Physarum* were termed HMG-1P, HMG-2P and HMG-14/17P, respectively. In addition to the 4 predominant proteins, a few minor polypeptides were also seen in the HMG preparation, but they were qualitatively and quantitatively variable, and therefore considered not to be the HMG proteins.

Protein HMG-14/17P seems to be identical with the acid-soluble non-histone protein of *Physarum* amoebae, called AS, which has an amino acid composition typical of HMG proteins [20]. This small HMG-like protein comigrates in SDS-gel systems with *Physarum* histone H2A, but may be separated from the histone by column chromatography on Bio-Gel P-100 [20] or hydroxyapatite (unpublished).

3.2. Distribution in chromatin

The HMG protein of vertebrates are characterized by preferential association with transcribed sequences in chromatin [2]. To determine whether or not the intragenomic localization of *Physarum* proteins identified as the HMGs is similar, we studied (i) protein composition of isolated chromatin fractions, which differ in transcriptional activity [15], and (ii) proteins solubilized from nuclei by DNase I digestion under conditions in which active genes are preferentially cleaved [21]. Such a procedure has frequently been used to locate chromosomal proteins with respect to the expressed regions of the genome.

Fig.2 shows electrophoretic profiles of proteins associated with the transcriptionally active and inactive chromatin fraction, and with unfractionated *Physarum* nuclei. It can be seen that all 3 HMG-like proteins were present almost exclusively in the transcriptionally active fraction. In particular, the active

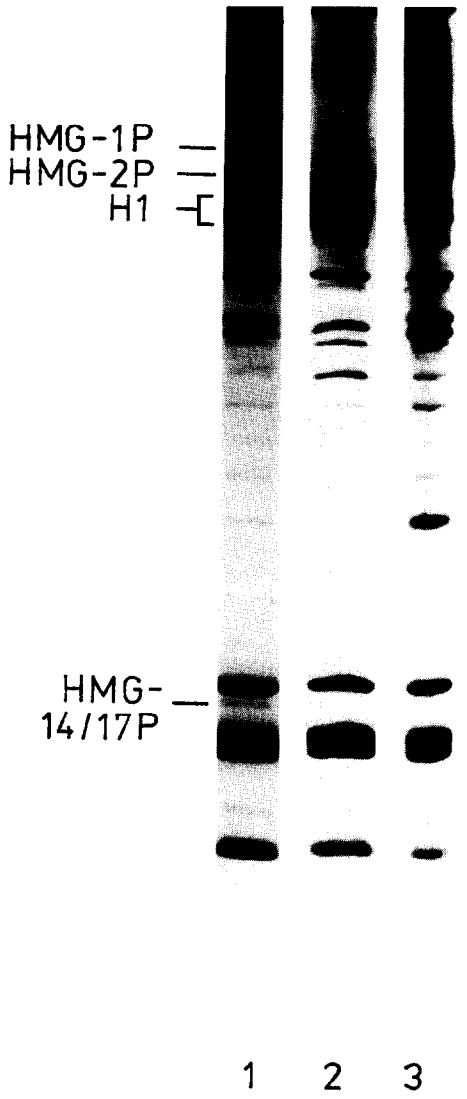

Fig.2. Electrophoretic patterns of proteins associated with: (1) transcriptionally active chromatin fraction, (2) inactive chromatin fraction, (3) unfractionated *Physarum* nuclei. Silver staining.

fraction contained great amounts of HMG-1P and HMG-2P, which were present at stoicheiometries comparable to those of the core histones. In contrast, the content of histone H1 was much higher in the fraction of inactive chromatin.

Proteins released from *Physarum* nuclei by mild DNase I digestion (<3% acid solubility of nuclear DNA) are shown in fig.3. Here again, the fraction most sensitive to the nuclease action contained all 3 HMG-like proteins. In this case, however, the

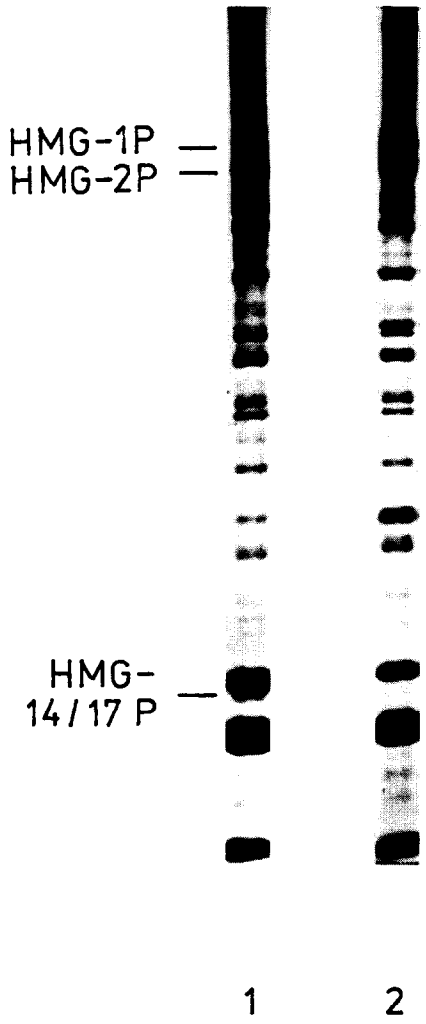

Fig.3. Electrophoretic patterns of: (1) proteins solubilized from *Physarum* nuclei by the light digestion with DNase I, (2) total nuclear proteins. Silver staining.

content of HMG-14/17P was significantly higher than that of the large HMGs.

The results obtained indicate that HMG-like proteins of *P. polycephalum,* like animal HMGs, may be involved in a process of transcriptional activation of genes.

4. DISCUSSION

The HMG proteins of various vertebrate species show a considerable degree of homology. In contrast, the available data indicate that HMG-like proteins of lower eukaryotes differ in physical and chemical properties from their vertebrate counterparts, and from each other. Our results indicate that this also holds for HMG-like proteins of *P. polycephalum*. These proteins are present in *Physarum* nuclei in amounts roughly comparable to those found in vertebrates [1,2], but differ in number (3 instead of 4) and in gel mobility from the HMG proteins of higher eukaryotes. *Physarum* proteins seem to be divergent from the known HMG-like proteins of other lower eukaryotes as well. However, with respect to their distribution in chromatin they behave like the HMG proteins of animal cells. They are present in great abundance in the transcriptionally active chromatin fraction, as well as being released from *Physarum* nuclei by the light DNase I digestion. Moreover, the active fraction contains reduced levels of histone H1 relative to the high-M_r HMG-like proteins, similar to data reported [22–24] for animal tissues. These findings strongly suggest that despite differences in molecular properties, HMG-like proteins of *P. polycephalum* may perform similar functions in the cell nucleus as animal HMGs.

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