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CHROMOSOMAL HMG PROTEINS OCCUR IN THREE EUKARYOTIC KINGDOMS

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SUMMARY

We report that yeast and wheat contain chromosomal proteins belonging to the high mobility group (HMG). This establishes that the HMG's are widely distributed throughout eukaryotes. We further suggest that prokaryotes may also contain HMG's.

The high mobility group (HMG) proteins have been found in a wide variety of mammalian tissues (1-4). They are chromosomal proteins and are distinguished by an unusual amino acid composition: Approximately half their residues are either basic or acidic and the basic and acidics nearly balance.

The HMG proteins are less abundant than the histones but are present in about 10^6 molecules per nucleus. Because of this high concentration Goodwin et al. (1, 2) have proposed that the HMG's are structural proteins of chromatin.

The HMG proteins are released from nuclei upon mild digestion with DNase I (5, 6). It has also been reported that DNase I preferentially digests structurally active portions of the genome (7-10) and the HMG proteins may therefore be located in such regions.

Aside from mammalian tissues, HMG's have been found in duck erythrocytes (6), trout testes (11, 12) and in insects (13, 14). However, they have not yet been reported in fungi or plants (1). Because of the possible role of the HMG's in chromatin structure and function, it is of evident importance to determine how widely HMG's are distributed. In the present paper we report that both wheat and yeast contain HMG proteins.

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MATERIALS AND METHODS

Chromatin from wheat germ (Triticum aestivum) (General Mills, Inc., Minneapolis, Minn.) was prepared either by the method of Simon and Becker (15) or from purified nuclei (16). The chromatin was washed with 0.075 M NaCl, 0.025 M EDTA and extracted with 0.35 M NaCl (2). The salt extracted proteins were made 2% w/v in trichloroacetic acid and the soluble proteins precipitated with acetone as described by Goodwin et al. (2). All steps were carried out at 0-4° and 0.1mM phenylmethyl-sulfonyl fluoride (PMSF) and/or 0.025 M $NaHSO_3$ were present in all solutions to inhibit proteolysis. No differences have been found when either inhibitor was used.

The putative wheat germ HMG's were analyzed by electrophoresis in polyacrylamide sodium dodecylsulfate (SDS) gels according to Thomas and Kornberg (17). HMG fractions were obtained by preparative electrophoresis in the same system. Bands in the preparative gels were visualized by phosphorescence (18) and cut out and eluted as described by Mardian (19). Amino acid analysis was carried out as previously described (20).

A prominent band appears in preparative and analytical gels of crude yeast histone (19, 21). This band was purified by preparative electrophoresis and its amino acid content determined as outlined above. Yeast (Saccharomyces cerevisiae) histone preparations were obtained either by a modification (19) of the method of Tonino and Rozijn (22) or from yeast nuclei prepared by a modification (19) of the method of Wintersberger et al. (23).

RESULTS

Figure 1a shows SDS gels of the proteins extracted from wheat germ chromatin with 0.35 M NaCl which are soluble in 2% w/v trichloroacetic acid. Four prominent bands appear which meet these preparative criteria for being HMG proteins. We have labeled them HMG a, b, c and d in an effort to avoid unwarranted interpretation concerning the correspondence of the wheat germ HMG's to HMG 1, 2, 3, etc. of calf thymus. The calf thymus HMG's are also shown in Figure 1a. The wheat germ bands labeled H1 in Figure 1a clearly are not HMG's as indicated by their amino acid content (24).

Figure 1b shows wheat germ HMG b isolated by preparative electrophoresis. Amino acid analysis of this protein is given in Table 1. Note that Asx plus Glx account for about 24 mole percent of the residues. Lysine plus arginine residues account for approximately 20 mole percent.

Figure 2 shows an acetic acid-urea gel (25) of the isolated yeast HMG compared to a gel of crude yeast histone. Amino acid analysis of the yeast HMG is given in Table 1. Note that Asx plus Glx account for approxi-

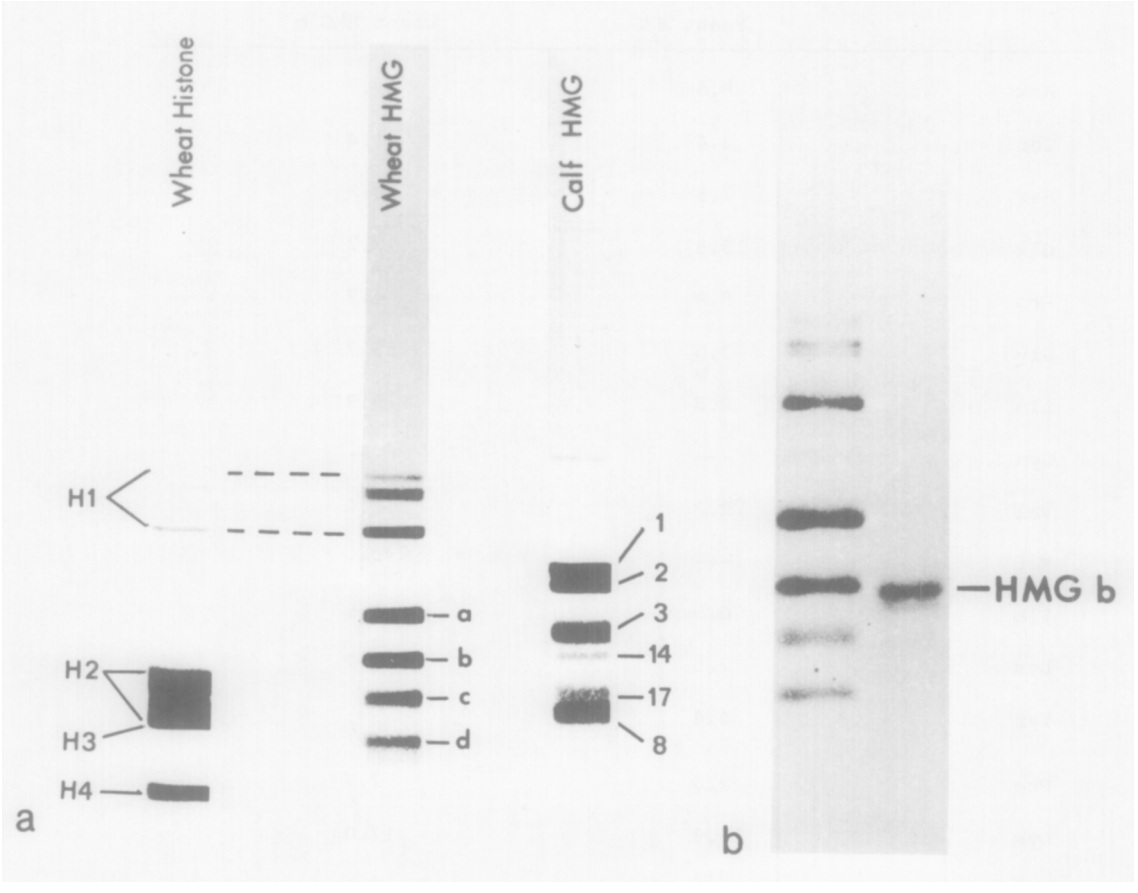

Figure 1 Sodium dodecylsulfate gels of (a) wheat histones, wheat HMG's and calf HMG's; (b) wheat HMG and isolated HMG b. Migration is from the top (-) toward the bottom (+).

mately 24 mole percent of the residues. Lysine plus arginine residues account for approximately 21 mole percent.

DISCUSSION

We have demonstrated the existence of chromosomal proteins from wheat and yeast with electrophoretic mobilities similar to those of HMG proteins from mammalian tissues. Moreover these wheat and yeast proteins have typical HMG amino acid compositions. We point out that while we have

Table 1. Amino acid analysis of HMG proteins from yeast and wheat

Amino Acid	Mole %	
	Yeast HMG	Wheat HMG b
Asx	8.5	11.7
Thr	3.4	1.4
Ser	7.5	8.3
Glx	15.6	12.7
Pro	5.9	4.7
Gly	3.6	13.7
Ala	8.8	15.9
Cys	---	---
Val	2.3	3.6
Met	---	---
Ile	6.5	1.5
Leu	7.5	2.2
Tyr	4.4	0.8
Phe	2.8	2.7
Lys	15.9	17.0
His	1.3	1.3
Arg	5.5	2.5
Typ	0.6*	Not determined

*estimated from absorbance spectrum

shown that these species contain HMG proteins, they may contain, in addition, HMG's other than the ones we have isolated. Our laboratory is currently investigating this possibility.

With the demonstration of HMG proteins in yeast and wheat, these chromosomal proteins have now been shown to exist in three of the four

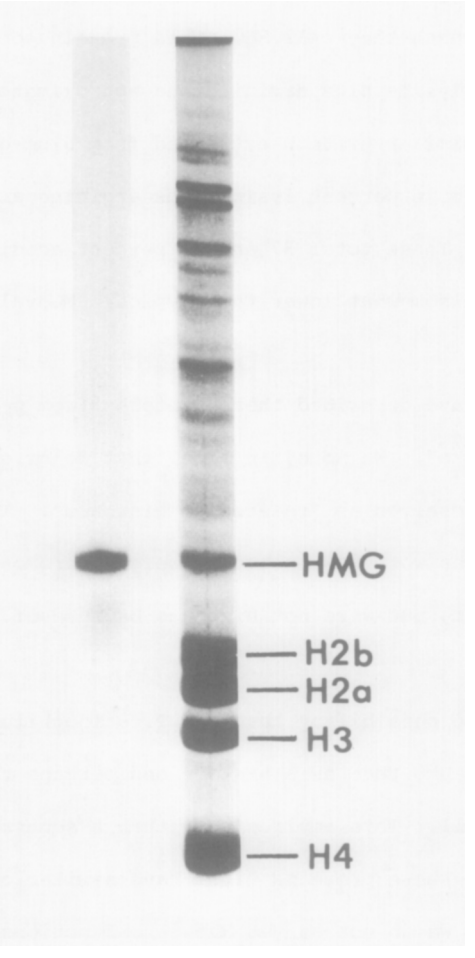

Figure 2 Acetic acid-urea gels of yeast crude histone preparation and purified yeast HMG. Migration is from the top (+) toward the bottom (-).

eukaryotic kingdoms. They therefore are widely distributed throughout eukaryotes although they have not yet been demonstrated in protista. We now ask: Do HMG's exist also in prokaryotes?

Searcy (26) has described a nucleoprotein from Thermoplasma acidophilum which contains approximately 20 mole percent Asx plus Glx and approximately 24 mole percent lysine plus arginine. This composition is typical of HMG's.

Rouviere-Yaniv and Gros (27) have reported a DNA-binding protein

from Escherichia coli which they call HU. This protein contains approximately 19 mole percent lysine plus arginine and approximately 18 mole percent Asx plus Glx. A similar protein extracted from blue-green algae contains approximately 19 mole percent lysine plus arginine and 16 mole percent Asx plus Glx (28, 29). These total 37 and 35 percent acidics plus basics respectively, which are somewhat lower than typical HMG values, but are still high.

The workers who have described these proteins from prokaryotes have termed them "histone-like". We note, in fact, that Johns et al. (30) described the HMG's themselves as "related to histones". These terms were used because the proteins were low molecular weight chromosomal proteins with high lysine content, but were not histones because of their high content of acid residues.

We now suggest the possibility that the T. acidophilum protein might be better classed as an HMG than as a histone and perhaps the other prokaryotic proteins as well. This implies more than a semantic preference. It suggests that all of these proteins might have similar or related functions. Since, however, we do not as yet know the functions of the HMG proteins, this suggestion is, of course, only speculative at the moment.

At the present time, precisely because the function is not known, we have no functional criteria for judging whether or not a given protein is an HMG. What all workers have done, ourselves included, is to call a chromosomal protein an HMG if it is of low molecular weight and has a characteristic amino acid composition including a high lysine content. By these criteria, we have now shown that the HMG proteins are universal throughout three eukaryotic kingdoms, and we have suggested that they may exist in all biological species.

ACKNOWLEDGEMENTS

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