High Mobility Group Proteins of *Saccharomyces cerevisiae*†

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ABSTRACT: The yeast *Saccharomyces cerevisiae* contains four proteins having amino acid compositions typical of the high mobility group (HMG) proteins. Three of these are eluted from chromatin by 0.35 M NaCl; one is not, but it is eluted by 0.25 N HCl. It follows that HMGs cannot, in general, be defined by extractability criteria. Gel mobilities and amino acid compositions indicate that yeast and animal HMGs have diverged markedly.

The high mobility group (HMG)[1] proteins occur in a variety of animal species (Goodwin et al., 1973, 1975; Watson et al., 1977; Wigle & Dixon, 1971; Smith & Stocken, 1973; Elgin & Bonner, 1972; Franco et al., 1977; Sterner et al., 1978; Johns et al., 1975) and in plants and yeast as well (Spiker et al., 1978). A number of studies have concluded that the HMGs are preferentially located in active regions of the genome (Vidali et al., 1977; Weisbrod & Weintraub, 1979; Levy et al., 1977a,b; Levy & Dixon, 1978a,b), although a recent report (Goodwin & Johns, 1978) has questioned this. In any case, the function or functions of the HMG proteins are not yet known.

The HMGs have been extracted from nuclei or chromatin by either 0.35 M NaCl (Goodwin et al., 1973, 1975) or 5% PCA (Goodwin et al., 1977a,b). They have unusual amino acid compositions. A large number of residues are basic, and a large number are acidic.

Of the many calf thymus HMGs, only four have been shown to be unique proteins (Goodwin et al., 1978a). The rest either are breakdown products or appear in such small amounts that it is difficult to say if they are breakdown products or not. Ubiquitin (Goldstein et al., 1975; Schlesinger et al., 1975) may be eluted from chromatin by 0.35 M salt (Watson et al., 1978; Walker et al., 1978), but it has an amino acid composition unlike that of the HMG proteins (Schlesinger et al., 1975) and it is doubtful if it should be included in that group.

In the present paper we report a study of the HMGs of the yeast *Saccharomyces cerevisiae*. The fungi in general constitute a separate kingdom in the five kingdom classification of Wittaker (1969) and are considered a separate major line of evolution alongside plants and animals. It is known that yeast contains at least one HMG (Spiker et al., 1978). Yet, to date, no one has attempted to characterize how many HMGs are in yeast (Petersen & Sheridan, 1978) or any other fungus or, for that matter, in any plant.

We find that *S. cerevisiae* contains four proteins having typical HMG amino acid compositions. Three of these may be eluted by 0.35 M NaCl. The fourth is not extractable by 0.35 M NaCl but may, however, be eluted by 0.25 N HCl. It follows that an HMG protein cannot be defined, in general, by criteria based on extractability. Our results also indicate that the HMGs show significant evolutionary divergence.

Materials and Methods

Yeast chromatin was prepared from pressed baker's yeast by the method of Mardian & Isenberg (1978a,b) which is a scaled up version of the method of Tonino & Rozijn (1966). Proteins were extracted either from freshly prepared chromatin or from chromatin first frozen overnight at −80 °C in 2.0 M sucrose, 5 mM Tris-HCl, pH 8.0, 1 mM $MgSO_4$, and 0.1 mM PMSF. No differences were observed in the proteins obtained by these two procedures. All steps were carried out at 0–4 °C, and 0.1 mM PMSF was added to all solutions to inhibit proteolysis.

HMGs were extracted according to the method of Goodwin et al. (1975). The purified yeast chromatin from 400 g of cells was suspended in 100 mL of 0.075 M NaCl, 0.025 M EDTA, 1 mM Tris-HCl, pH 7.5, and 0.1 mM PMSF and then centrifuged in a swinging bucket rotor for 10 min at 9000*g*. This was then repeated. The chromatin pellet was then suspended by one stroke of a tightly fitting Potter-Elvehjem homogenizer in 100 mL of 0.35 M NaCl, 1 mM Tris-HCl, pH 7.0, and 0.1 mM PMSF. It was stirred gently in the cold (2 °C) for 1 h. The suspension was centrifuged in a swinging bucket rotor for 10 min at 9000*g* to pellet the chromatin. The 0.35 M NaCl supernatant was poured off and maintained at 0 °C. The chromatin was extracted a second time for 30 min by using 50 mL of the same solution in the same manner. The combined 0.35 M NaCl extracts were made 2% in Cl_3AcOH by the addition of 87.6% Cl_3AcOH while being stirred rapidly. (The concentration of the Cl_3AcOH was determined by measuring the index of refraction). After the addition of the Cl_3AcOH, the suspension was stirred for 5 min to allow the complete precipitation of LMG proteins to occur. The suspension was centrifuged in the swinging bucket rotor for 10 min at 9000*g*. The supernatant was made 0.01 M in β-mercaptoethanol, and then with rapid stirring 2.25 mL of concentrated (29.2% w/v) NH_4OH was added, followed by the rapid addition of 3 volumes of cold acetone.

Histones were obtained from the chromatin pellet which had been extracted by 0.35 M NaCl. The pellet was suspended in 50 mL of glass-distilled water with one stroke of a Potter-Elvehjem homogenizer, and 50 mL of 0.5 N HCl was then added. It was stirred gently at 2 °C for 1 h and then centrifuged in a swinging bucket rotor for 20 min at 9000*g*. Eight volumes of cold acetone was added to the supernatant.

The HMG and histone precipitates were collected by centrifugation in a swinging bucket rotor for 10 min at 9000*g*. The HMG precipitate was washed twice with cold acidified acetone (6 volumes of acetone to 1 volume of 0.1 N HCl) and 3 times with cold acetone and then dried under vacuum. The histone precipitate was washed 3 times with cold acetone, pelleted at 9000*g* for 10 min in a swinging bucket rotor, and dried under vacuum.

The individual yeast HMGs were isolated by using preparative electrophoresis on acetic acid–2.5 M urea–polyacryl-

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[1] Abbreviations used: HMG, high mobility group; LMG, low mobility group; PMSE, phenylmethanesulfonyl fluoride; PCA, perchloric acid; Cl_3AcOH, trichloroacetic acid; EDTA, ethylenediaminetetraacetic acid; $NaDodSO_4$, sodium dodecyl sulfate.

0006-2960/80/0419-2236$01.00/0