On the presence of HMG proteins in yeast

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Two polypeptides antigenically related to mammalian HMG1/2 have been detected in the yeast *Saccharomyces cerevisiae*. One exhibits an electrophoretic mobility in the range of mammalian HMG1/2 whereas the second polypeptide comigrates with yeast HMG S4. Evidence for the presence of HMG14/17 in yeast has not been obtained by immunological or nuclease digestion-based methods, although their presence cannot be excluded.

High-mobility group protein *Chromatin* *Antibody* *Micrococcal nuclease* *DNase I*
(Saccharomyces cerevisae)

1. INTRODUCTION

High-mobility group (HMG) non-histone chromosomal proteins may be classified into two categories. The smaller ones, HMG14 and HMG17, are thought to be associated with nucleosomal core particles in active chromatin, although the evidence for their precise role is controversial [1]. Less defined proposals have been made for the function of HMG1 and HMG2. Several facts have led to the proposal that they bind to linker DNA: (i) a proportion of these proteins is released by short nuclease digestion; (ii) under these conditions, they seem to be attached to short polynucleotides; (iii) they bind to long nucleosomes (review [2]).

Both classes of HMG proteins have been unequivocally found in the vertebrates investigated so far [3]. Putative HMG proteins have been described in insects [4–6], plants [7,8], protozoa [9] and yeast [7,10]. The study of HMG proteins in yeast offers some advantages in determining whether they are associated with active chromatin, because most of the yeast genes are, at least potentially, active. To this end we have characterized protein fractions prepared from yeast chromatin and show that in these fractions there are electrophoretic bands that react with antibodies raised against mammalian HMG1.

2. MATERIALS AND METHODS

Yeast chromatin was prepared from commercial pressed baker's yeast as described [11]. Yeast nuclei were obtained from exponentially growing cells of *Saccharomyces cerevisiae* CECT 1383, following the procedure of Lohr and Ide [12].

To study the enzymatic release of proteins, nuclei were suspended in 0.25 M sucrose, 10 mM NaCl, 3 mM $MgCl_2$, 1 mM $CaCl_2$, 10 mM Tris-HCl pH 7.4 to a concentration of about 0.5 mg DNA/ml. DNase I (10 U/ml) or micrococcal nuclease (20 U/ml) was then added and the mixture incubated at 37°C for the appropriate times. To the influence of endogenous nucleases, some incubations were carried out without added enzyme. Digestions were stopped by chilling the tubes on ice and the nuclei recovered by centrifugation at 3000 × *g* for 10 min at 4°C. Supernatants were made to 0.25 M HCl and 8 vols cold acetone added to the clarified solutions to precipitate the proteins.

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Saline extractions of chromatin or nuclei with 0.14 or 0.35 M NaCl were carried out according to Goodwin et al. [13].

Chicken erythrocyte HMG1, HMG2 and HMG14 were prepared by 5% perchloric acid extraction, followed by chromatography on CM-Sephadex G-25 [14].

Electrophoresis of proteins was carried out in acetic acid-urea-polyacrylamide slab gels [15]. After electrophoresis, some of the samples were transferred to nitrocellulose filters by electroblotting at 7.5 V/cm for 15 min (HMG14) or 90 min (remaining proteins). All transfers were made in duplicate; one of the filters was stained with 0.1% amido black in 10% acetic acid-45% methanol and the second incubated with anti-HMG antibodies essentially according to Durán et al. [16]. Filters were soaked in saline buffer (0.15 M NaCl, 50 mM Tris-HCl, pH 7.4) and washed with incubation buffer (0.15 M NaCl, 50 mM Tris-HCl, pH 7.4, 3% bovine serum albumin, 0.05% Nonidet P-40, 0.02% NaN_3) for 2 h at 37°C. The incubation with antibody (at 5 μg protein/ml) was performed in incubation buffer at 37°C overnight. The filters were then washed with saline buffer at 1 M NaCl and with saline buffer again. The filters were incubated with 0.25 μCi per slot of ^{125}I-protein A (37 μCi/mg, Amersham) in incubation buffer at 37°C for 2 h, followed by washing with saline buffer, with 1 M NaCl and once more with saline buffer. Filters were then dried and autoradiographed at −70°C with MAFE RP-X1 film.

Antibodies were elicited in rabbits by injecting purified calf thymus HMG1 or HMG2 essentially as in [17]. The antibodies were purified by immunoabsorption [18] and shown to react only with the corresponding proteins from calf thymus, rat liver and chicken erythrocyte chromatin.

3. RESULTS

Fig. 1 shows that the major polypeptides extracted with 0.35 M NaCl from nuclei of exponentially growing cells do not coincide with the most important proteins extracted from chromatin of quiescent cells, termed S1, S2, S3 and S4 by Weber and Isenberg [10]. The 3 bands that appear in our nuclear extracts will be further referred to as C1, C2 and C3, in order of increasing mobility in acetic acid-urea-polyacrylamide gels. The mobilities of

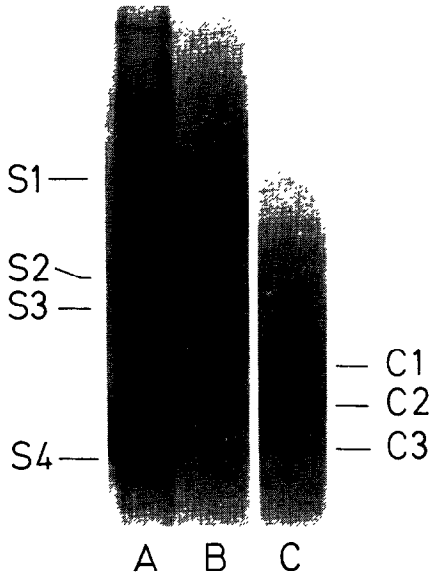

Fig.1. Acetic acid-urea-polyacrylamide gel electrophoresis of proteins extracted from nuclei or chromatin of *S. cerevisiae*. (A) 0.35 M NaCl extract from baker's yeast chromatin, according to Weber and Isenberg [10]. (B) Proteins released from nuclei of exponentially growing cells by micrococcal nuclease digestion (5 min). (C) 0.35 M NaCl extract from nuclei of growing cells.

C3 and S4 are very similar, but it is difficult to decide whether they are identical. The other 2 polypeptides seem to be present in the extracts from baker's yeast chromatin, but only as minor components visible in overloaded gels such as that of fig.1A. All the C polypeptides are also partially extracted with 0.14 M NaCl (not shown).

Typical yields of S proteins are in the range of 3% with respect to histones, as expected for HMG proteins [19]. The level of C polypeptides in growing cells is at least 10-fold higher.

These proteins are released from nuclei by nuclease digestion. Fig.1 shows that C1 is preferentially released after micrococcal nuclease digestion, although C2 and C3 are also present in digests, especially after long digestion periods. DNase I and endogenous nucleases (not shown) apparently have analogous effects in the preferential release of C1, followed by C2 and C3, although the reaction is very slow in digestions with endogenous nucleases. Taking advantage of the relative abundance of C1 in short-time digests, we have determined the amino acid composition of a

Table 1

Amino acid composition of yeast C1, S2 and S4 proteins

Amino acid	C1	S2[a]	S4[a]
Asx	10.4	9.9	9.5
Thr	5.6	5.5	5.6
Ser	15.4	9.6	8.0
Glx	11.5	12.5	11.7
Pro	7.9	n.d.[b]	n.d.[b]
Gly	14.4	13.5	11.4
Ala	7.6	9.1	9.5
Cys	–	–	–
Val	4.4	8.3	4.8
Met	–	–	–
Ile	3.2	0.6	3.0
Leu	4.7	8.5	6.7
Tyr	–	0.7	0.7
Phe	2.2	1.7	5.7
His	4.2	7.8	4.6
Lys	6.6	8.8	14.1
Arg	2.0	3.9	5.1

[a] From Weber and Isenberg [10]
[b] Not determined

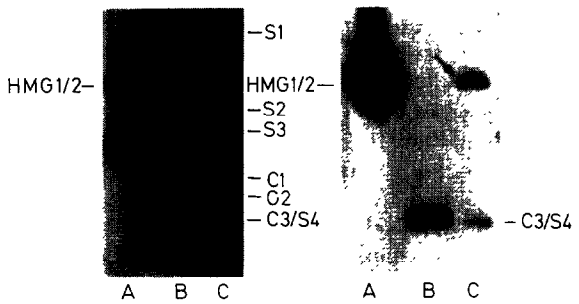

Fig.2. Immunoreactivity of C and S proteins from *S. cerevisiae* against calf anti-HMG1 antibodies. Proteins were electrophoresed and blotted as described in section 2. (A) HMG1/2 from chicken erythrocyte. (B) Proteins extracted with 0.35 M NaCl from pressed baker's yeast chromatin. (C) Proteins extracted with 0.35 M NaCl from nuclei of exponentially growing cells. Left: amido black-stained blot. Right: autoradiogram. The position of C0 protein is indicated by the arrow.

C1-enriched preparation such as that shown in fig.1B. The results in table 1 show that the content of basic amino acids is lower than expected for HMG proteins.

On the other hand, we have tested the immunoreactivity of C1–C3 and S1–S4 towards antibodies elicited by calf thymus HMG14/17 and calf thymus HMG1. Neither C nor S polypeptides cross-react with anti-HMG14/17 antibodies (not shown). Fig.2 shows that a distinct reaction of two polypeptides with anti-HMG1 takes place. One of the polypeptides corresponds to S4 of Weber and Isenberg and is found in 0.35 M NaCl extracts from chromatin of quiescent cells. The second only appears in extracts from growing cells, its mobility being in the range of calf thymus HMG1/2. This polypeptide must be strongly antigenic, since it is not detectable in amido black-stained strips and reacts considerably over background. A much weaker band also appears in the region of C3.

4. DISCUSSION

The major polypeptides released from yeast nuclei by nuclease digestions do not resemble HMG proteins. A C1-enriched preparation posesses too low a proportion of basic amino acids to be considered a typical HMG (table 1). In fact, the amino acid analysis of C1 is very similar to that of S2, whose HMG-like nature was ruled out by Weber and Isenberg [10] based on compositional criteria. Moreover, the partial extractability of C proteins in 0.14 M NaCl also argues against their HMG-like nature. The absence of cross-reaction with antibodies against calf thymus HMGs is a less definite proof, since HMGs from yeast and animals may have diverged markedly [10]. Taking into account that the level of C proteins is high in genetically active nuclei, while they are present to a lesser extent in quiescent chromatin, we believe that they may represent a number of loosely bound proteins associated in some way with active chromatin or nascent RNA. Proteins associated with nuclear ribonucleoprotein particles may be plausible candidates.

The function of HMGs, as well as that of other non-histone proteins in chromatin, remains unknown. For this reason, it has been suggested that antibodies can serve as tools to identify these components [20]. Recently, Vanderbilt and Anderson [21], using this approach, failed to detect any protein in perchloric acid extracts from yeast. The present results, however, indicate that a relationship exists between mammalian HMG1 and at least one protein in yeast nuclei, namely that marked with an arrow in fig.2, further referred to as C0.

A second polypeptide, S4, is also immunoreactive, although to a small degree. It may be a second antigenic HMG protein or represent a proteolytic peptide derived from C0. Two reasons would favour the second possibility. First, proteolytic activity of yeast, especially in stationary cells, is very high [22]; this could explain why the C0 band is only detectable in growing cells. Second, HMG proteins are very susceptible to proteolytic degradation [23]. Whatever the interpretation, we must emphasize that these results clearly show the existence of HMG proteins in yeast that belong to the HMG1/2 class.

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