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Amino-terminal sequence of a *Saccharomyces cerevisiae* nuclear protein, NHP6, shows significant identity to bovine HMG1

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Several nonhistone chromatin proteins (NHPs) have been isolated from *Saccharomyces cerevisiae* nuclei. They have molecular masses and amino acid compositions typical of the high mobility group (HMG) proteins from higher eukaryotic cells. Polyclonal antisera raised against two of the NHPs have been used in immunoblots of proteins from subcellular fractions of yeast to show that the NHPs are indeed nuclear. In addition, the amino-terminal amino acid sequences of several of the NHPs were determined. Importantly, the amino-terminal sequence of one of the proteins, NHP6, has significant (60%) identity with a stretch of amino acids in calf thymus HMG1.

High mobility group protein; Amino acid sequence; Antibody; Chromatin; (*Saccharomyces cerevisiae*)

1. INTRODUCTION

Eukaryotic nuclear DNA is packaged into a nucleoprotein complex called chromatin. While the major protein components of chromatin, the histones, have a well defined role in chromatin structure, the functions of most nonhistone chromatin proteins are unclear. One class of low molecular mass nonhistone proteins is released from chromatin by 0.35 M salt or perchloric acid [1]. They are called the high mobility group (HMG) proteins. Vertebrate cells contain four major HMG proteins which share the property of containing over 25% basic amino acids as well as over 25% acidic amino acids [2]. It has been postulated that the HMG proteins have roles in a variety of cellular processes, including transcription and DNA replication [3,4], but the data are somewhat controversial [5–7].

In order to determine conclusively the functions of HMG proteins in the cell, we have undertaken a combined genetic and biochemical approach using the yeast *Saccharomyces cerevisiae*. Several HMG-like proteins have previously been identified in yeast on the basis of their extractibility by 0.35 M salt and their highly acidic/highly basic amino acid compositions [8]. However, since HMG protein function is uncertain, the best criterion for establishing a relationship to the prototypic calf thymus HMG proteins would be amino acid sequence homology. This has not been demonstrated for any HMG-like protein from yeast. In this report we present the first amino acid sequence data for HMG-like proteins from *S. cerevisiae* and show that at least one has significant amino acid identity to a sequence in the calf thymus HMG1 protein.

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2. MATERIALS AND METHODS

2.1. *Extraction of nonhistone chromatin proteins (NHPs)*

For the identification of the NHPs, nuclei were isolated from cells of the *S. cerevisiae* strain NNY [9] using the Percoll gradient procedure of Potashkin [10] with minor modifications. The purified nuclei were then split into four equal fractions. The proteins in one fraction were solubilized by boiling in SDS. A second fraction was extracted with 0.35 M NaCl essentially

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as described [8]. 5% perchloric acid was used to extract proteins from the third sample according to the protocol of Peterson and Sheridan [11] but the differential acetone precipitations were omitted. Histones were extracted from the last sample using 0.25 N HCl and acetone precipitation [8].

2.2. *Purification and analysis of NHPs*

To purify individual NHPs, 10 l of NNY cells were used to make nuclei [10] except the Percoll step was omitted. NHPs were then extracted from this fraction with 0.35 M NaCl [8], suspended in SDS loading buffer [1], boiled 2 min and then electrophoresed on preparative SDS-polyacrylamide gels. After electrophoresis, the proteins were visualized by staining 10 min in aqueous 1% Coomassie brilliant blue R and destaining in several changes of water. To elute each protein, gel slices containing individual NHPs were cut out and put at 65°C overnight in 3 ml of 0.1% SDS, 10 mM sodium phosphate (pH 7.5).

For amino acid composition analysis and amino-terminal protein sequencing, each purified protein sample was concentrated and then dialyzed against five changes of 0.02% SDS. Amino acid composition analysis was done on a Durum D-500 amino acid analyzer after hydrolysis of 2–10 nmol of sample in 6 N HCl at 110°C for 24 h in evacuated sealed tubes. The amino-terminal amino acid sequences were determined from approx. 0.5 nmol of each purified protein by the Gas-Phase Protein Sequencing Service, Baylor College of Medicine, Houston, TX.

2.3. *Western blot analysis of cellular fractions*

Purified NHP2 and NHP4 were each used to raise antibodies in rabbits [12]. NNY cells were used to make ribosomes [13], mitochondria [14] or nuclei [10]. The proteins in each of these fractions were electrophoresed in triplicate on a 15% SDS-polyacrylamide gel. The fractionated proteins were subsequently transferred to nitrocellulose using a Bio-Rad Trans-Blot cell. One blot was stained. The others were washed in Blotto [15], and then incubated either with anti-NHP2 antiserum diluted 1/60 in Blotto or with a 1/60 dilution of anti-NHP4 antiserum. After 2 h, the filters were washed 3 times in Blotto and then incubated for 2 h with goat anti-rabbit peroxidase-conjugated antisera diluted 1/2000 in Blotto. Following three 20 min washes in TBS, the bound antibodies were visualized using a Bio-Rad horseradish peroxidase substrate kit. The incubations were done at room temperature with shaking.

3. RESULTS AND DISCUSSION

3.1. *Identification of yeast HMG-like proteins by amino acid composition*

The prototypic calf thymus HMG proteins are extracted from chromatin by either 0.35 M NaCl or 5% perchloric acid [1]. To identify HMG-like proteins in *S. cerevisiae*, yeast chromatin was similarly extracted using these two procedures. Both extraction protocols resulted in an enrichment of a subset of proteins from the total nuclear proteins of yeast (fig.1A, lane 1). The major proteins which are solubilized both by perchloric acid

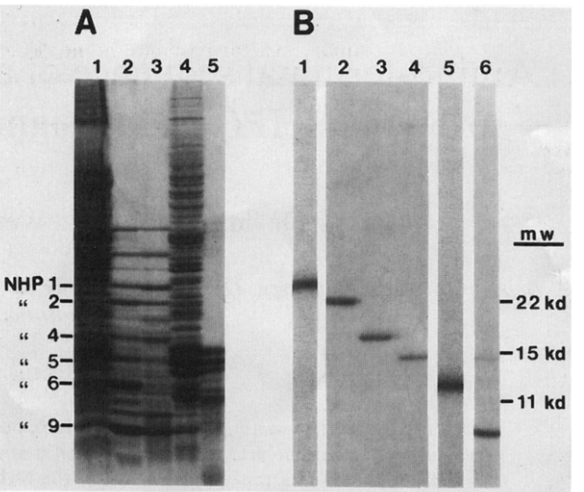

Fig.1. Electrophoretic analysis of crude and purified NHPs. Proteins were electrophoresed on a 15% SDS-polyacrylamide gel and stained with Coomassie brilliant blue R. (A) *S. cerevisiae* nuclei were extracted with (1) SDS, (2) 0.35 M NaCl, (3) 5% perchloric acid, or (4) 0.25 N HCl, which solubilizes predominantly histones. Lane (5) contains calf thymus histones. (B) The lanes contain gel purified proteins: (1) NHP1, (2) NHP2, (3) NHP4, (4) NHP5, (5) NHP6 and (6) NHP9.

(fig.1A, lane 3) and by 0.35 M salt (fig.1A, lane 2) and whose molecular masses are less than 30 kDa are the best candidates for yeast HMG-like proteins. These proteins have been labelled NHP (nonhistone chromatin protein) 1–9 in fig.1A.

Another criterion for identifying HMG-like proteins is their unusual amino acid composition [2]. Thus, the six NHPs were purified by preparative gel electrophoresis and subjected to amino acid composition analysis; the results are presented in table 1. We conclude that NHP2, NHP4, and NHP6 are the best candidates to be HMG proteins. Like the calf thymus HMG proteins, they have high (20%) and nearly equal percentages of basic and acidic amino acids. However, the overall amino acid compositions of NHP2, NHP4 and NHP6 differ from those of the calf HMG proteins (not shown). Of the other NHPs, one, NHP1, is not basic enough and two of them, NHP5 and NHP9, are not acidic enough to be considered prime HMG protein candidates.

On the basis of size and amino acid composition, Weber and Isenberg [8] have identified four HMG-like proteins in yeast, S1, S3, S4 and HMGa. Electrophoresis of our NHPs on the acid-

Table 1

Amino acid compositions (in mol%) of NHPs and other putative yeast HMG proteins

	NHP2[a]	S1[b]	NHP4[a]	S3[b]	NHP6[a]	S4[b]	NHP1[a]	NHP5[a]	NHP9[a]	HMGa[b]
Asx[c]	9.6	7.8	14.2	6.6	10.7	9.5	14.4	5.7	9.8	8.5
Thr	2.5	5.9	6.1	7.3	7.2	5.6	5.9	7.0	6.1	3.4
Ser	6.4	10.4	7.8	9.9	4.3	8.0	7.7	10.7	3.2	7.5
Glx[d]	10.9	9.7	10.5	10.2	12.2	11.7	10.3	10.2	2.6	15.6
Pro	4.9	–[e]	4.2	–	6.4	–	5.8	3.2	2.3	5.9
Gly	6.5	7.7	10.3	5.6	7.8	11.4	13.1	7.4	5.5	3.6
Ala	8.1	9.9	9.9	10.8	11.4	9.5	11.7	12.1	15.2	8.8
Cys	–	0.7	–	0	–	0	–	–	–	–
Val	10.2	5.5	5.0	5.5	5.0	4.8	5.4	4.9	2.0	2.3
Met	0.1	0	0.2	0	0	0	0.4	0	0	–
Ile	5.0	3.8	4.5	5.7	2.5	3.0	2.5	6.0	1.6	6.5
Leu	6.6	6.5	5.2	5.0	6.7	6.7	4.0	6.4	5.1	7.5
Tyr	1.4	2.0	0.7	4.9	2.7	0.7	0.8	2.6	0.7	4.4
Phe	2.4	2.8	0.4	4.7	2.4	5.7	1.5	1.6	1.8	2.8
His	3.0	4.9	1.1	7.2	0.7	4.6	1.6	1.6	12.2	1.3
Lys	19.2	19.2	11.3	12.2	12.4	14.1	10.1	15.1	24.2	15.9
Arg	3.2	3.5	8.6	4.7	7.5	5.1	5.1	5.2	7.6	5.5
Acidics[f]	20.5	17.5	24.7	16.8	22.9	21.2	12.4	15.9	24.7	24.1
Basics[g]	22.4	22.7	19.9	16.9	19.9	19.2	31.8	20.3	15.2	21.4
Lys/Arg	6.0	5.5	1.3	2.6	1.7	2.8	3.2	2.9	1.9	2.9

[a] This paper
[b] [8]
[c] Asx, aspartic acid + asparagine
[d] Glx, glutamic acid + glutamine
[e] –, not determined
[f] Acidics, Asx + Glx
[g] Basics, Lys + Arg

urea gel system used by Weber and Isenberg gives a pattern of proteins similar to that which they observed (not shown). The amino acid compositions of their proteins and our NHPs are compared in table 1; none of the proteins paired on the basis of electrophoretic mobility have very similar compositions. Thus, we have to conclude that the HMG-like proteins isolated by Weber and Isenberg and our NHPs may be different proteins. However, we cannot rule out the possibilities that strain differences, differences in the physiological state of the yeast at the time of protein isolation or differences in the protein isolation procedures could account for the observed discordance in amino acid compositions.

3.2. *Yeast HMG-like proteins are nuclear*

The NHPs appear to be nuclear proteins since we have found them in salt or perchloric acid extractions of yeast nuclei prepared by a number of different procedures including differential centrifugation [16] and centrifugation in Percoll gradients [10,17]. However, purified yeast nuclei are often contaminated by mitochondria and ribosomes [16]. Therefore, to establish more convincingly that the NHPs are nuclear proteins, polyclonal antibodies were raised against two of the purified proteins, NHP2 and NHP4. These antisera were used in immunoblots of nuclear, mitochondrial and ribosomal proteins from yeast (fig.2). Anti-NHP2 antiserum reacts only with the nuclear fraction and the most intense band migrates at the same position as purified NHP2. The lower band on the anti-NHP2 immunoblot is presumably a proteolytic fragment of NHP2 since it is not always seen in these experiments. The anti-NHP4 antiserum reacts strongly with a protein in the nuclear fraction which migrates at the same position as NHP4. There is also a weaker reaction with a much larger protein in the ribosomal frac-

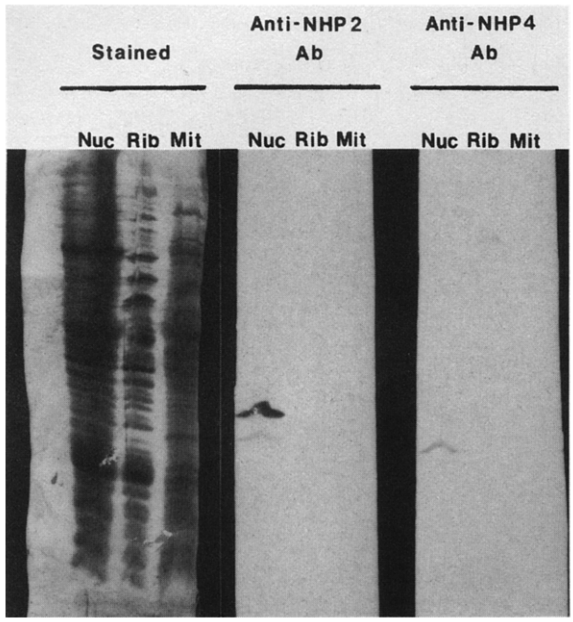

Fig.2. Western blot analysis showing that NHP2 and NHP4 are nuclear (Nuc) proteins, not ribosomal (Rib) or mitochondrial (Mit) proteins).

tion. Since the signal in the ribosomal fraction is much weaker and reacts with a differently sized protein, we conclude that it is due to a minor reactivity in the polyclonal antiserum. These data demonstrate that NHP2 and NHP4 are nuclear proteins; it is likely that the other NHPs are also nuclear.

3.3. *Amino acid sequence identity between NHP6 and calf thymus HMG1*

The best evidence that the NHPs are related to higher eukaryotic HMG proteins would be a demonstration of significant amino acid sequence homology between an NHP and an HMG protein. Therefore, to obtain protein sequence for such a comparison, the amino-terminal amino acid sequences of each NHP were determined (not shown). The amino-terminus of NHP4 was blocked to amino acid sequencing.

When the partial amino acid sequences of the NHPs were compared to the sequences of the HMG proteins from higher eukaryotes [18,19], only one protein, NHP6, matched any HMG protein at more than six residues. The similarity between NHP6 and the HMG1 family of proteins is striking (fig.3); almost 60% of the amino-terminal

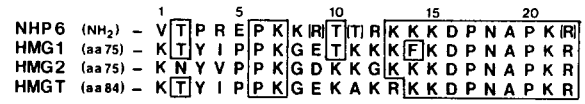

Fig.3. Amino acid sequence identities between NHP6, calf thymus HMG1 and HMG2 and trout HMGT. The amino-terminal amino acid sequence of NHP6 is aligned with part of the amino acid sequences of calf thymus HMG1 [19] and HMG2 [18] and trout HMGT [19] starting at amino acids 75, 75 and 84, respectively. Identities between NHP6 and the other sequences are boxed. Tentative amino acid assignments are in parentheses.

twenty-two amino acids of NHP6 are identical to a stretch of amino acids in the calf thymus HMG1 protein. Furthermore, the matches are also found in the sequences of the calf HMG2 and trout HMGT proteins. However, the region of identity is not at the amino-termini of HMG1, HMG2 or HMGT. Perhaps the first 75 amino acids found in HMG1 are not required for NHP6 to function like an HMG protein in *S. cerevisiae*. Another possibility is that NHP6 is a stable proteolytic byproduct of a larger protein. Finally, this significant identity between NHP6 and HMG1 may reflect a conserved function of part of each protein and the rest of the sequence of the two proteins may not be similar. To distinguish among these possibilities, we will be determining the sequence of the rest of NHP6 by cloning its gene.

The National Biomedical Research Foundation protein database was searched for any sequences homologous to the sequences of the NHPs using the FASTP homology search program of Lipman and Pearson [20]. The sequences of NHP1, NHP2, and NHP9 did not share significant identities with any proteins in the database. However, the sequence of NHP5 was identical to the yeast histone H2B [21] sequence at 17 of 19 amino acids. Since NHP5 migrates at about the same position as histone H2B on SDS-polyacrylamide gels (fig.1A) and has significant sequence identity to H2B, we conclude that NHP5 is probably H2B.

When the NHP6 sequence was compared to the protein database, there were four histone H1 proteins, two HMG17 proteins, a wheat histone H2A1 and a sea urchin histone H2B among the top twenty matches found (HMG1 and HMG2 are not in the database). Examination of these matches revealed that they were each due to a short stretch

of similarity containing 7–10 identities, mainly in the lysine-rich middle portion of the NHP6 sequence. Interestingly, the sequences in the H1 and HMG17 proteins which are similar to the NHP6 sequence are the same ones which other investigators [3,22] found to be similar between HMG17 and histones H1 and H5. It has been postulated that these short sequences may be involved in DNA binding. We are investigating this for NHP6.

We have shown that yeast has several HMG-like proteins, at least one of which has some significant amino acid identity to the prototypic calf thymus HMG1 protein. We are currently cloning the genes encoding the NHPs in order to begin genetic studies on NHP function.

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