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p23, a novel mammalian nucleic acid-binding protein with homology to the yeast ribosomal protein YL43

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When separating perchloric acid-soluble proteins from cell cultures and tissues by chromatography on single stranded DNA agarose columns, a novel mammalian protein with extreme affinity for DNA was isolated. Cellular localization, amino acid composition and the N-terminal sequence suggest that the protein is a ribosomal protein with extensive sequence homology to the ribosomal protein, YL43, from *Saccharomyces cerevisiae*.

Ribosomal protein; Mammal; PCA-soluble protein

1. INTRODUCTION

Extraction of cells and tissues with 5% perchloric acid (PCA) selectively solubilize histone H1, the HMG group of proteins and a small number of unidentified proteins [1]. Both H1 and the HMG proteins have an unusual amino acid composition comprising nearly 50% charged residues which might explain their solubility properties. One of the most prominent, unidentified, PCA-soluble protein present in all cell types, is a protein with an apparent molecular weight of 23 kDa. The protein has an extreme affinity for single- and double-stranded DNA. This can be employed to purify the protein by chromatography on single-stranded DNA agarose.

2. MATERIALS AND METHODS

2.1. *Materials*

Single-stranded (ss) DNA was purchased from Bethesda Research Laboratories.

2.2. *Preparation of perchloric acid extracts*

Extraction of cells and tissues was carried out as in [2].

2.3. *Preparative acetic acid–urea gel electrophoresis*

This was carried out as in [2].

2.4. *Isolation of p23*

DNA agarose containing 0.5–1.0 mg ss DNA from calf thymus was packed in a column with dimensions (0.6 × 22 cm) and washed with 1.0 mM Tris–HCl, pH 7.5. PCA-soluble proteins from 15 g liver was dissolved in 1.0 mM Tris–HCl containing 0.05 M NaCl and applied on the column. The proteins were eluted with 50 ml of a linear salt gradient from 0.2–0.6 M NaCl in Tris buffer at a flow rate of 0.5 ml/min. The column was subsequently washed with 20 ml 1.0 mM Tris–HCl containing 2 M NaCl.

For preparative purposes, materials from 80 g rat liver or 2×10^9 cells were extracted with 5% PCA and separated by preparative gel electrophoresis. The p23-containing fraction was applied on the ss DNA column in 1.0 mM Tris–HCl/0.05 M NaCl, pH 7.5. The column was washed with buffer containing 0.6 M NaCl before p23 was eluted with 50 ml of a linear gradient from 0.6–2.0 M NaCl.

2.5. *Amino acid analysis*

Protein samples were hydrolyzed for 24 h with 6 M HCl in evacuated tubes at 108–110°C and analyzed on a Biotronic LC 5000 amino acid analyzer. No correlations have been made for hydrolytic losses.

2.6. *Sequence determination*

Purified protein was sequenced by automated Edman degradation using a pulsed phase sequenator (Applied Biosystems 477A). Resulting PTH amino acid derivatives were identified using an on-line-connected PTH-analyzer (Applied Biosystem 120A).

2.7. *SDS polyacrylamidegel electrophoresis*

This was performed in accordance to Laemmli [3].

2.8. *Subcellular fractionation and isolation of ribosomes*

This was carried out as in [4,5].

3. RESULTS

3.1. *Isolation and amino acid analysis of p23*

PCA-soluble proteins can be separated by chromatography on ss DNA agarose. H1 and the HMG proteins are eluted from the column between 0.1 and 0.6 M NaCl, while p23 elutes at 0.75–0.95 M NaCl (Figs. 1 and 2). Due to the large amount of H1 relative to p23 in the PCA extract, preparative gel electrophoresis of PCA-extracted material was performed prior to chromatography on a ss DNA column in the isolation procedure for p23.

The amino acid composition of p23 from three differ-

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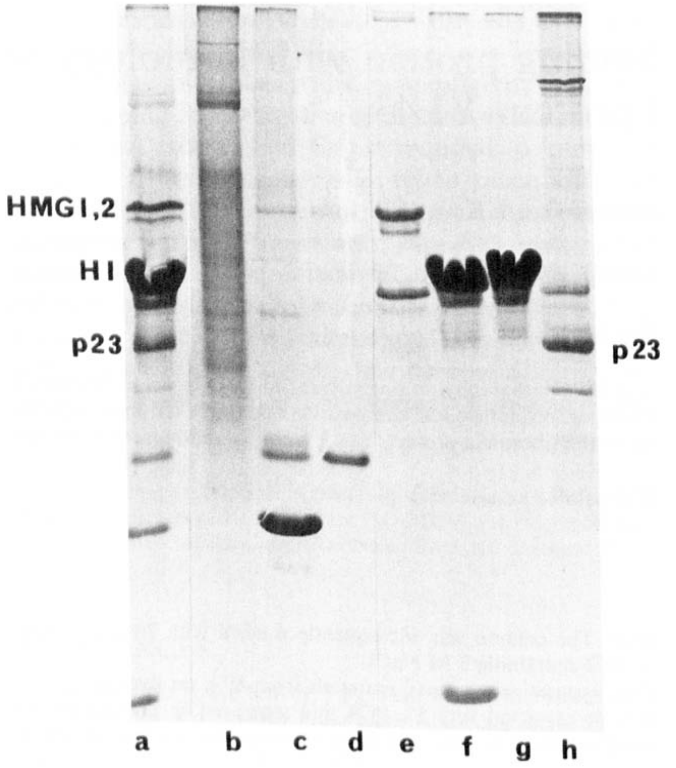

Fig. 1. Acetic acid–urea polyacrylamide gel electrophoresis of PCA-soluble proteins from rat liver fractionated on a ss DNA agarose column. A Coomassie blue-stained gel is shown. (a) Rat liver PCA extract; (b) proteins eluted at 0.240 M NaCl; (c) proteins eluted at 0.340 M; (d) proteins eluted at 0.380 M; (e) 0.450 M; (f) proteins eluted at 0.550 M; (g) proteins eluted at 0.6 M; and (h) proteins eluted at 2 M NaCl.

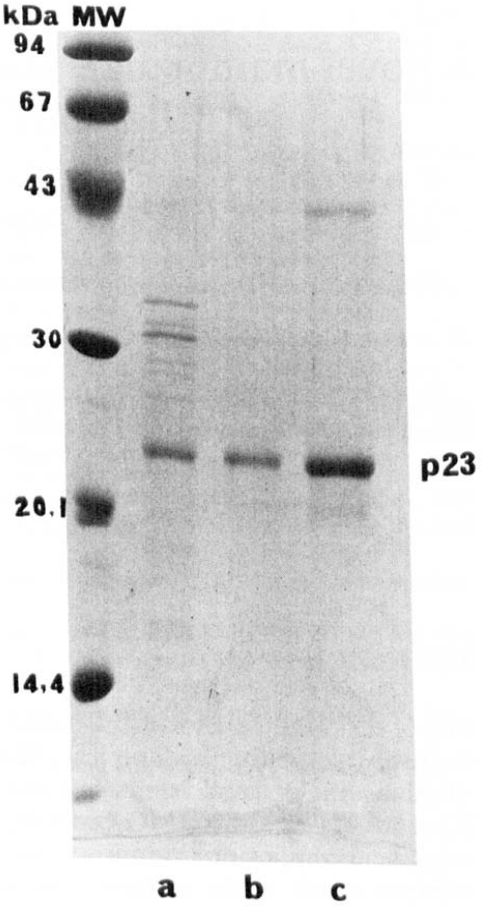

Fig. 2. SDS–polyacrylamide gel electrophoresis of a crude fraction of p23 fractionated on a ss DNA agarose column. The proteins were eluted with a linear gradient from 0.6–2.0 M NaCl. A Coomassie blue-stained gel is shown. (a) A crude preparation of p23 obtained after preparative gel electrophoresis; (b) proteins eluted between 0.700–0.800 M NaCl; (c) proteins eluted between 0.800–0.950 M NaCl.

ent mammalian species (human, rat and mouse) was determined (Table I) and shows that p23 is characterized by a high content of basic amino acids and alanine. The protein is quite hydrophilic and contains more than 30% charged or hydrophilic residues.

3.2. *Amino terminal sequence of p23*

p23 from rat liver was subjected to sequence analyses by automated Edman degradation, and the N-terminal sequence is shown in Fig. 3. Two amino acids in the p23 sequence are unidentified. In position four, the yield of the lysine derivative was low, but an extra peak appeared indicating a modified lysine residue. This modified lysine residue might represent a methylated form of lysine.

The EMBL Data Library and The Swissprot database was searched for any sequences homologous to the sequence of p23, and the result reveals that p23 has extensive sequence similarities with the N-terminal end of the ribosomal protein YL43 from *Saccharomyces cerevisiae* as shown in Fig. 3. Comparison of the two sequences shows 75% identity in 28 amino acid overlap in the N-terminal end. There are three conservative and two non-conservative replacements. N-terminal analyses (5 amino acids) of p23 from HeLa cells showed 100% identity with p23 from rat liver. Also in this case an extra peak appeared in position four together with the lysine derivative.

3.3. *Subcellular localization*

When isolating a cytoplasmic and a nuclear fraction prior to the PCA extraction, p23 seems to be distributed

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          *  10                  20        *
p23   A-K-S-K-N-H-T-T-H-N-Q-S-R-K-X-H-R-N-G-I-K-K-P-R-X-Q-R-Y
YL43  A-K-S-K-N-H-T-A-H-N-Q-T-R-K-A-H-R-N-G-I-K-K-P-K-T-Y-K-Y
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Fig. 3. N-Terminal amino acid sequence of p23 and the yeast ribosomal protein, YL43. Asterisks indicate non-conservative replacements.

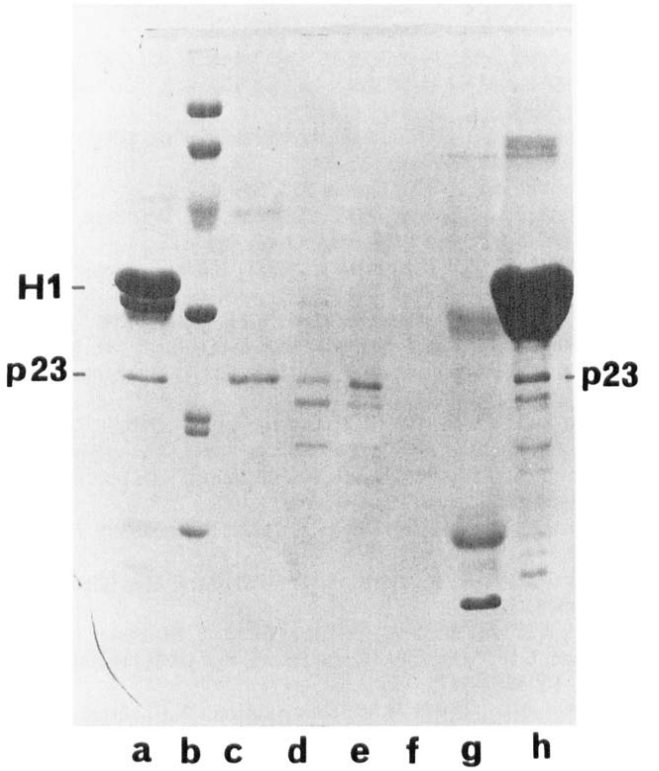

Fig. 4. SDS–polyacrylamide gel electrophoresis of PCA-soluble proteins in different subcellular fractions of rat liver cells. A Coomassie blue-stained gel is shown. (a) PCA extract from rat liver; (b) molecular weight markers; (c) purified p23; (d) microsomal fraction; (e) lysosomal fraction; (f) mitochondrial fraction; (g) soluble fraction; (h) nuclear fraction.

between the cytoplasm and the nucleus. As seen in Fig. 4, subcellular fractionation of rat liver hepatocytes shows that the protein is present in the nuclear as well as in the microsomal and the lysosomal fraction. The

Table I

The amino acid composition of p23 from different species

	HeLa	EAT	Rat liver	YL43
Asx	5.5	5.5	5.5	8.5
Thr	2.9	2.3	2.3	6.5
Ser	5.3	5.2	7.0	3.5
Glx	8.6	9.3	8.6	2.4
Pro	9.0	10.6	8.1	5.1
Gly	6.6	7.4	5.1	5.8
Ala	16.2	15.9	16.1	13.9
Cys	ND	ND	ND	ND
Val	3.6	3.4	3.8	2.8
Met	1.2	1.6	2.3	–
Ile	2.3	2.2	2.2	1.7
Leu	4.8	4.5	5.3	5.4
Tyr	0.80	0.87	0.87	2.3
Phe	1.7	1.8	1.9	2.0
His	2.3	3.0	3.2	10.0
Lys	19.3	19.4	20.4	22.4
Arg	6.7	6.2	7.2	7.7

The values represent mol % (residues per 100).
ND, not detected

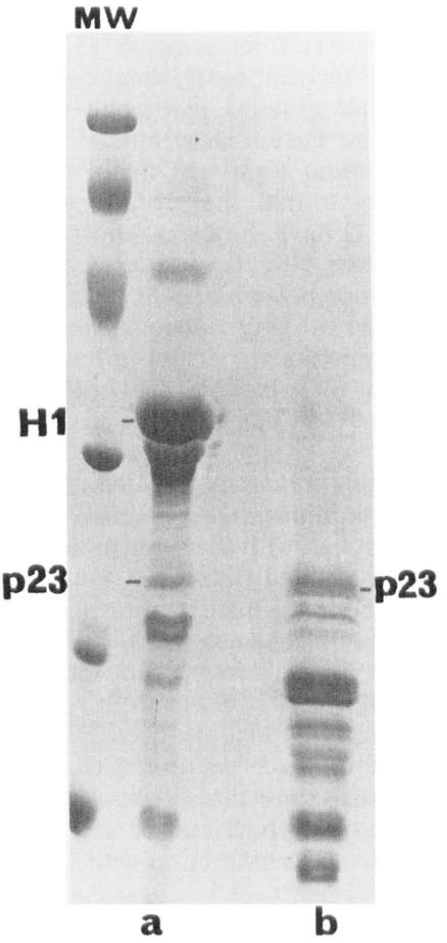

Fig. 5. SDS–polyacrylamide gel electrophoresis of PCA-soluble proteins from rat liver and isolated ribosomes. A Coomassie blue-stained gel is shown. (a) Rat liver; (b) isolated rat liver ribosomes.

microsomal and lysosomal fraction is, however, highly cross-contaminated and the results suggest that p23 might be a ribosomal protein. When extracting purified ribosomes with 5% PCA, a protein with the same molecular weight as p23 was present in addition to a number of low molecular weight proteins (Fig. 5), indicating that p23, indeed, is a ribosomal protein in mammals. p23 is present in all mammalian species so far examined.

4. DISCUSSION

Ribosomes from eukaryotic organisms, including yeast, contain a large number of proteins [6,7]. Several proteins from the small and large subunit of *Saccharomyces cerevisiae* have been identified [7]. Yeast ribosomal proteins range in size from about 3.5 to about 44 kDa [8]. Complete or N-terminal sequences have been reported for a number of these proteins [9–11]. The amino acid sequence of four ribosomal proteins from *Saccharomyces cerevisiae* have also been deduced from the nucleotide sequences of cloned genes [12–14]. More than 80 proteins have also been isolated from rat liver

ribosomes and characterized in terms of molecular weight and amino acid composition [15,16]. N-Terminal amino acid sequences have been determined for a number of these proteins. Although most of the sequenced proteins have a large number of basic amino acids at the amino terminus, there are no sequence repeats to suggest that the proteins are related [16]. Otaka et al. [17] have, however, shown that the yeast ribosomal protein, YS9, has sequence homology to the rat liver ribosomal protein S8 [16]. There is also homology between the rat liver ribosomal protein, L37, and the yeast *S. cerevisiae* ribosomal protein, SC-Y55 [16], and between the rat liver ribosomal protein, L9, and the recently cloned *Saccharomyces cerevisiae* protein, YL9A [18].

p23 is probably a mammalian homologue to YL 43, a protein present in the large ribosomal subunit from *S. cerevisiae*. A protein with the same molecular weight as p23 is found in isolated ribosomes, but a large proportion is also found in the nuclei indicating that p23 might be part of a pre-ribosomal particle. The amino acid composition of p23 is, however, not identical to any of the reported ribosomal proteins from rat liver [15]. The significant N-terminal identity between YL43 and p23 may reflect a conserved function in the ribosome. The present results also show that ribosomes contain a small number of proteins which can be separated from the remaining proteins by extraction with 5% PCA.

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