Volume 257, number 2, 247–250 FEB 07766 November 1989

Prothymosin α is an evolutionary conserved protein covalently linked to a small RNA

Tatyana Makarova, Nickolay Grebenshikov, Cesiy Egorov, Andrey Vartapetian and Alexey Bogdanov

Belozersky Laboratory of Molecular Biology and Bioorganic Chemistry, Moscow State University, Moscow 119899, USSR

Received 5 September 1989

A 13 kDa protein, covalently linked to a small RNA from the cytoplasm of mouse cells, was studied. Sequence analysis of its tryptic peptides revealed that the RNA-linked protein is identical to prothymosin α. Very similar RNA-protein complexes were identified in human, bovine and yeast cells. Tryptic peptide maps of ^{125}I-labelled RNA-linked proteins of diverse origin demonstrated their marked similarity, thus indicating high evolutionary conservation of prothymosin α from yeast to man.

Protein-RNA complex; Prothymosin α; Evolutionary conserved protein

1. INTRODUCTION

RNA genomes of a number of animal and plant viruses are known to possess at their 5′-termini a covalently linked viral protein (review [1]). Recently, we have found a cellular protein covalently bound to a small RNA in the cytoplasm of mouse cells. This 13 kDa protein was shown to be attached to an RNA molecule of about 20 nucleotides long, apparently to its 5′-end [2]. In this paper we present evidence that the RNA-linked protein is identical to prothymosin α, an extremely acidic protein previously identified as a possible precursor of an immunomodulating peptide [3]. Very similar RNA-protein complexes were identified in a number of eukaryotic cells, including human, bovine and yeast. Analysis of peptide maps showed that the RNA-linked proteins of diverse origin are structurally very similar, which testifies to high evolutionary conservation of prothymosin α. Moreover, identification of prothymosin α in yeast suggests that, apart from its immunomodulating activity, it probably provides some more general function in the cell.

2. MATERIALS AND METHODS

The source of covalent RNA-protein complex was the RNA preparation from mouse Krebs II ascites carcinoma cells, obtained as described in [2]. High-molecular weight RNA was eliminated by precipitation with 2 M lithium chloride and 2 M urea; supernatant was ethanol precipitated and chromatographed on DEAE-cellulose column. The complex was eluted with 0.3 M sodium chloride.

For the final purification of the RNA-protein complex and for the separation of tryptic peptides the material was fractionated by reversed-phase HPLC using 'Ultrasphere-octyl' 4.6 × 150 mm column at 40°C in 0.1% trifluoroacetic acid. A linear gradient of isopropanol of 0 to 60% was used for RNA-protein isolation, and of 0 to 30% for peptide fractionation.

Protein labelling with the ^{125}I Bolton and Hunter reagent was described in [2]. Hydrolysis of the RNA-bound protein with TPCK-trypsin (0.01 mg/ml, Worthington) was performed in 100 mM Tris-HCl, pH 8, for 2 h at 37°C. Two-dimensional peptide mapping was performed on cellulose-coated thin layer sheets. First dimension: electrophoresis in acetic acid/formic acid/water, 15:5:80, v/v; second dimension: chromatography in butanol/pyridine/acetic acid/water, 25:10:4:12, v/v.

Automated Edman degradation was carried out with an Applied Biosystems model 470A/120A gas-phase sequencer. The search for homologous protein sequences in the NBRF protein databank was performed with the 'MicroGenie' programme (Beckman).

Correspondence address: A. Vartapetian, Belozersky Laboratory of Molecular Biology and Bioorganic Chemistry, Moscow State University, Moscow 119899, USSR

3. RESULTS

3.1. *The RNA-linked protein is prothymosin α*

In order to purify the covalent RNA-protein complex from mouse Krebs II cells, the preparation of the cytoplasmic RNA was used, which has been separated from the non-covalently bound proteins by repeated phenol-SDS extraction, as described earlier [2]. RNA fractionation (see section 2) has permitted one to obtain a preparation of the complex suitable for the analysis by reversed-phase HPLC. The HPLC-purified material (fig.1A, fractions 34–35) proved to be homogeneous, as judged by the electrophoresis in 8% PAG, and comigrated with the ^{125}I-labelled authentic RNA-protein complex (fig.3, lane 7).

Direct sequencing of the protein moiety of the complex failed, presumably due to the blocked N-terminus. Therefore, the RNA-linked protein was hydrolyzed with trypsin and the peptides were fractionated by reversed-phase HPLC (fig.1B). Peptides I–V were

Published by Elsevier Science Publishers B.V. (Biomedical Division)
00145793/89/$3.50

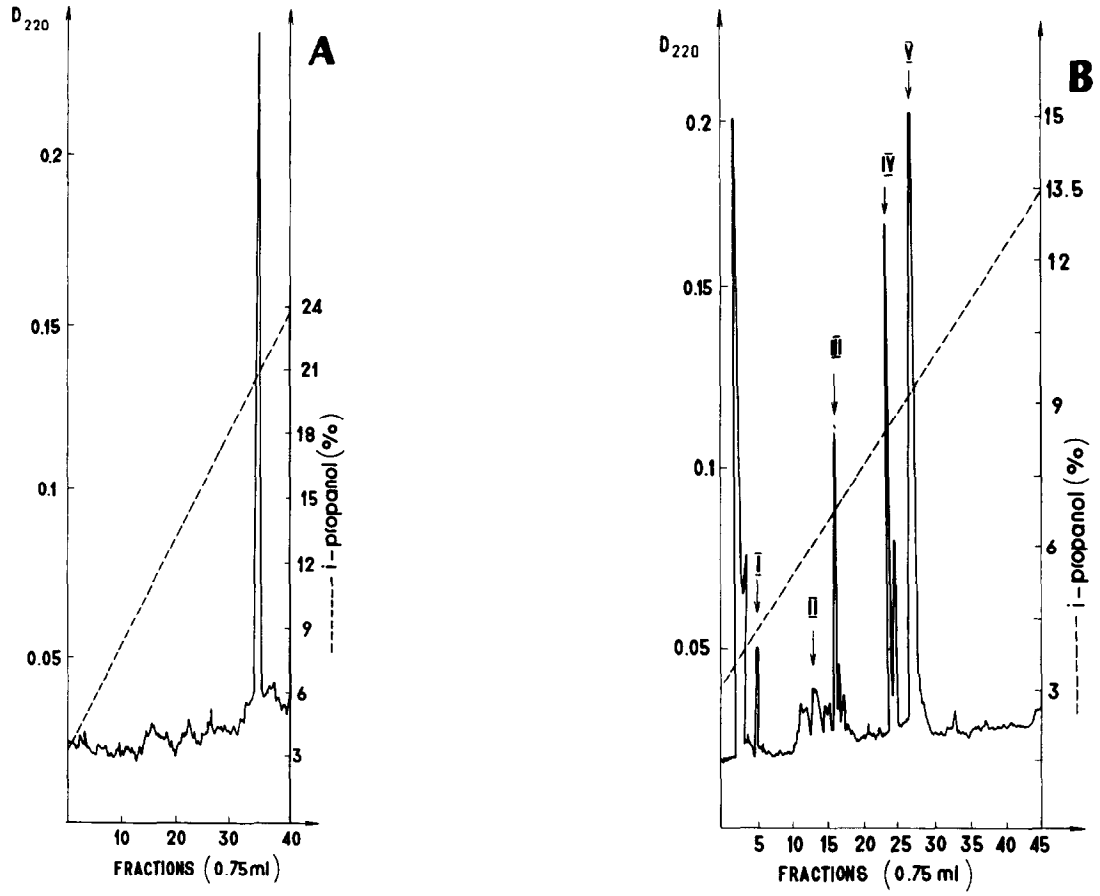

Fig.1. (A) Purification of the covalent RNA-protein complex from mouse cells by HPLC. (B) Fractionation of tryptic peptides of the RNA-linked protein by HPLC.

chosen for sequence analysis. The resultant amino acid sequences of these peptides, derived from the mouse RNA-linked protein, are shown in table 1. The peptides turned out to be extremely acidic and did not contain aromatic or sulfur-containing amino acids. Peptide V was not sequenced to its very C-terminus because of its length and presence of long stretches of glutamic acid residues. Peptide IV was resistant to the Edman degradation, most likely due to the blocking group at its N-terminus. This suggests that it represents the amino-terminus of the RNA-bound protein.

Amino acid sequence of peptide III was used for a computer-assisted search for homologous protein sequences in the protein sequence database. The only protein identified, which contained this sequence, was rat prothymosin α, an 111 amino acid long protein, which indeed has a blocked (acetylated) N-terminus [3]. Moreover, prothymosin α contains the amino acid se-

Table 1

The primary structures of tryptic peptides of the mouse RNA-linked protein[a]

NN	Sequence
I	^{15}D^{b}LK^{17}
II	^{90}VAEDDEDDDVETK^{102}
III	^{21}EVVEEAENGR^{30}
IV	blocked N-terminus $^{(1-14?)}$
V	^{31}DAPANGNAQNEENGEQEADNEVDEEEEEGGEEEEEEE^{67}...

[a] Numbers indicate the position of the corresponding peptide in the rat prothymosin α sequence (see fig.2)

[b] A small amount of alanine was also detectable at this step

```
                10                  20
SDAAVDTSSEITTKDLKEKKEVVEE
        30                  40                  50
AENGRDAPANGNAQNEENGEQEADN
                60                  70
EVDEEEEEGGEEEEEEEEGDGEEED
        80                  90                  100
GDEDEEAEAPTGKRVAEDDEDDDVE
                110
TKKQKKTDEDD
```

Fig.2. Amino acid sequence of tryptic peptides of the mouse RNA-linked protein (underlined) and of rat prothymosin α [9]. Basic residues are shown in bold type.

quences of all other sequenced peptides of the RNA-linked protein, see fig.2. These two proteins are of identical size, and comparison of HPLC profiles of their tryptic peptides reveals marked similarity [10]. Thus, we conclude that the cytoplasmic protein from mouse cells covalently bound to an RNA is prothymosin α.

3.2. *Prothymosin α is an evolutionary conserved protein*

The RNA preparation from human RD cells, bovine thymus and yeast *S. cerevisiae* cells was purified from the non-covalently bound proteins and radiolabelled with the ^{125}I Bolton and Hunter reagent known to label proteins, but not RNA. The RNA fractionation by PAGE demonstrated that the ^{125}I-labelled material identical to the mouse prothymosin α-RNA complex was present in other cells, fig.3. Sensitivity of the

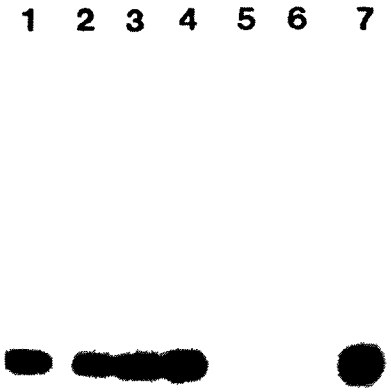

Fig.3. ^{125}I-labelled covalent RNA-protein complexes from human (1), mouse (2), bovine (3) and yeast (4) cells are very similar. Lanes 5 and 6, yeast RNA-protein complex treated with RNase T2 and proteinase K, respectively. An autoradiograph of 8% PAG is shown. Lane 7, HPLC-purified mouse RNA-protein complex stained with methylene blue.

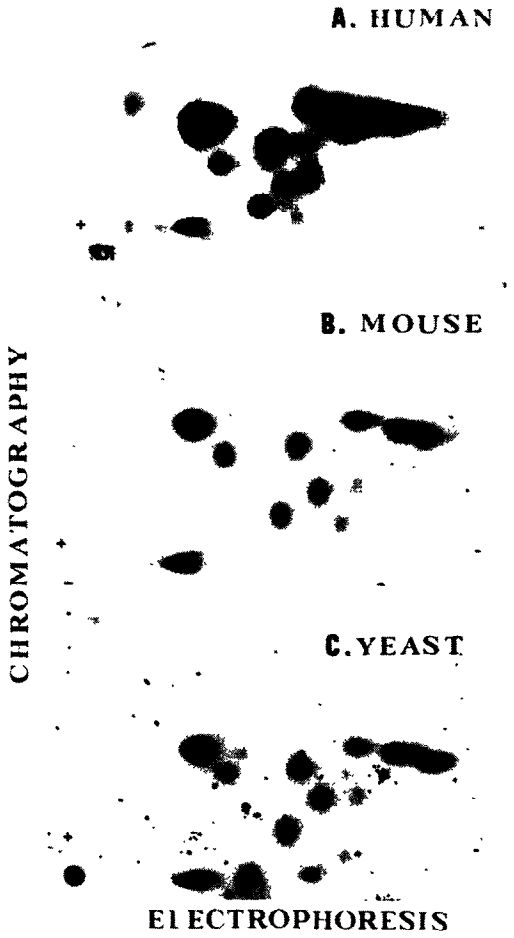

Fig.4. ^{125}I tryptic peptide maps of the RNA-linked proteins from human (A), mouse (B) and yeast (C) cells.

detected band to treatment with proteinase K and RNase T2 (fig.3, lanes 5 and 6) indicated the existence of both RNA and protein moieties in the material under study. Identification of the yeast RNA-protein complex is particularly interesting. It should be mentioned, however, that its amount in the yeast cells is approx. 1000 times smaller than that in mammalian cells.

^{125}I-labelled RNA-linked proteins from human, mouse (prothymosin α) and yeast cells were digested with trypsin, and two-dimensional fractionation of the resultant ^{125}I-peptides was performed on thin-layer cellulose plates. Fig.4 demonstrates that the maps of RNA-linked proteins are nearly identical not only in human and mouse cells, but also in yeast. This suggests that the RNA-linked protein in diverse cells is identical or very similar to prothymosin α, and that the structure of this protein is highly conserved from yeast to man.

4. DISCUSSION

Recently we have identified, in the cytoplasm of mouse cells, a 13 kDa protein covalently bound to the 5′-end of a small RNA molecule [2]. The amino acid

sequence of several tryptic peptides presented in this paper demonstrates that this protein is prothymosin α. Presence of the covalently linked RNA was not discovered for prothymosin α earlier. Prothymosin α, an exceptionally acidic protein, was claimed to possess immunomodulating activity [3], and was classified as a thymic hormone. However, prothymosin α lacks some features, which are characteristic of peptide hormones: it is expressed in a wide variety of tissues [4] and lacks a N-terminal signal sequence [4,5]. The data on its subcellular distribution are contradictory, indicating either nuclear [6,7], or cytoplasmic localization [11]. Our study definitely indicates that prothymosin α, when bound to an RNA, is located preferentially in the cytoplasm [2]. It cannot be excluded, however, that the protein-bound RNA can affect the subcellular localization of prothymosin α.

The primary structure of prothymosin α from a number of mammalian cells has been established, and minor amino acid substitutions were observed [4,5,8–10]. Our finding that prothymosin α from higher eukaryotes is well-conserved is consistent with these data. However, as far as we know, this is the first report demonstrating the presence of prothymosin α in an organism other than vertebrates. It seems obvious that the immunomodulating activity of prothymosin α is not its sole function. High evolutionary conservation of prothymosin α, from yeast to man, could possibly testify to its fundamental role in the cell.

Acknowledgements: We are grateful to Drs E. Koonin and E. Yankilevskaya for the participation in some experiments, to Dr A. Metlitskaya for the yeast RNA preparation, to Drs A. Mankin and V. Agol for many stimulating discussions.

REFERENCES

[1] Vartapetian, A.B. and Bogdanov, A.A. (1987) Progr. Nucleic Acid Res. Mol. Biol. 34, 209–251.
[2] Vartapetian, A.B., Makarova, T.N., Koonin, E.V., Agol, V.I. and Bogdanov, A.A. (1988) FEBS Lett. 232, 35–38.
[3] Haritos, A.A., Blacher, R., Stein, S., Caldarella, J. and Horecker, B.L. (1985) Proc. Natl. Acad. Sci. USA 82, 343–346.
[4] Eschenfeldt, W.H. and Berger, S.L. (1986) Proc. Natl. Acad. Sci. USA 83, 9403–9407.
[5] Goodall, G.J., Dominguez, F. and Horecker, B.L. (1986) Proc. Natl. Acad. Sci. USA 83, 8926–8928.
[6] Gomez-Marquez, J. and Segade, F. (1988) FEBS Lett. 226, 217–219.
[7] Watts, J.D., Cary, P.D. and Crane-Robinson, C. (1989) FEBS Lett. 245, 17–20.
[8] Panneerselvam, C., Wellner, D. and Horecker, B.L. (1988) Arch. Biochem. Biophys. 265, 454–457.
[9] Frangon-Lazaridis, M., Clinton, M., Goodall, G.J. and Horecker, B.L. (1988) Arch. Biochem. Biophys. 263, 305–310.
[10] Economou, M., Seferiadis, K., Frangon-Lazaridis, M., Horecker, B.L. and Tsolas, O. (1988) FEBS Lett. 233, 342–346.
[11] Tsitsiloni, O.E., Yialouris, P.P., Sekari-Pataryas, K. and Haritos, A.A. (1989) Experientia 45, 332–334.