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Thesaurin a, the major protein of *Xenopus laevis* previtellogenic oocytes, present in the 42 S particles, is homologous to elongation factor EF-1α

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We have purified in SDS *X.laevis* thesaurin a (M_r 50000) which is part of the 42 S storage particles. Its N-terminal amino acid is blocked and several peptides obtained by V8 protease treatment were purified and sequenced. As expected from one of the functional roles of the 42 S particles (tRNA binding, protection against deacylation and exchange with the ribosome), the amino acid sequence of thesaurin a was found to be closely related to that of the elongation factor EF-1α. We suggest that all three proteins involved in 5 S RNA and tRNA storage in previtellogenic oocytes, TFIIIA, thesaurin a and thesaurin b, have a dual function: storage and a role in transcription or in protein synthesis.

Elongation factor; Thesaurin a; Sequence homology; Amino acid sequence; tRNA-containing particle; 5 S RNA-containing particle; (*Xenopus laevis*, Oocyte)

1. INTRODUCTION

In previtellogenic oocytes of *Xenopus laevis* large amounts of 5 S RNA and tRNA are stored in nucleoprotein particles [1,2], the thesaurisomes [3]. The larger of these particles sediments at 42 S and contains, in addition to tRNA and 5 S RNA [1,2], two proteins with apparent M_r values of 50000 (thesaurin a) and 40000 (thesaurin b) [4,5]. Thesaurin a is the most abundant protein in the cytoplasm of previtellogenic oocytes, amounting to about 7% of the protein content of these cells [4]. Thesaurin a binds tRNA and thesaurin b binds 5 S RNA [4,5]. The 42 S particles are directly involved in protein synthesis [6,7]. Not only do the particles exchange tRNA with the ribosomes [6,7] but they also sustain a low-rate polymerization of amino acids [8], which may be the consequence of the storage conditions provided to aminoacyl tRNA in the particles [8]. These conditions are such that aminoacyl tRNA cannot be discharged in the particles [9]. This suggests that some kind of relationship might exist between thesaurin a and EF-1α [6,7], which exclusively binds aminoacyl tRNA [10] and protects it against deacylation [10]. Thesaurin a shares immunological cross-reactivity with *Artemia salina* EF-1α [7]. In this paper we report that the amino acid sequence of *X. laevis* thesaurin a is homologous to that of EF-1α from *A. salina* [11], *Homo sapiens* [12] and *X. laevis* (Krieg, P. and Melton, D., unpublished).

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Abbreviations: EF-1, elongation factor 1; TF, transcription factor; PAGE, polyacrylamide gel electrophoresis; V_o and V_t, void and total volume

2. EXPERIMENTAL

The 42 S particles present in previtellogenic

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oocytes of *X. laevis* were purified by centrifugation into a linear sucrose gradient (15–30%, w/v, 14 ml, containing 50 mM Tris-HCl, pH 7.6, 25 mM KCl, 5 mM $MgCl_2$, 0.1 mM DTT) at 280000 × *g* for 4 h in Beckman SW 41 Ti rotor. The particle-containing fractions were treated with 0.1 vol. of 500 mM Tris-HCl, pH 6.8, 10 mM EDTA, 20% (w/v) SDS, 10% (v/v) 2-mercaptoethanol, and heated for 70 s at 100°C. After a low-speed centrifugation the samples (2 ml, containing about 25 μg of protein) were injected on a preparative TSK 3000 SWG column (600 × 21.5 mm). This column is designed for gel filtration of large samples by high-performance liquid chromatography (HPLC). Elution was carried out at a flow rate of 4 ml/min with 30 mM Hepes, 10 mM imidazole, pH 7, 200 mM sodium acetate, 0.1 mM DTT, 0.1% (w/v) SDS (room temperature).

Thesaurin a purified by HPLC was concentrated 10-fold in Centricon 10 and digested by staphylococcal V8 protease [13] (Miles) for 1 h at 37°C at a thesaurin a/V8 ratio of 3.4/1 (w/w). 1 vol. thesaurin a in HPLC buffer was added to 1 vol. of 125 mM Tris-HCl, pH 6.8, 1 mM EDTA, 0.1% SDS (w/v), heated for 70 s at 100°C before addition of 2 vol. V8 protease in the same buffer, but containing 10% (v/v) glycerol. Proteolysis was stopped [13] and the samples were loaded on a 10.4% polyacrylamide gel [14]. Slices of Coomassie blue stained gels containing a single V8 protease-derived peptide were rinsed (3 × 1 h) in bidistilled water, electroeluted (Biotrap) in 50 mM Tris-HCl, 340 mM glycine, pH 8.8, 2 mM EDTA, 0.1 mM DTT, 0.1% (w/v) SDS for 4 h at 70 mA and 180 V. The electroeluted peptides were extensively dialyzed (15 h and 2 × 2 h) in a Spectrapor 2000 dialysis bag against 1 l of 10 mM $(NH_4)_2CO_3$, 0.01% (w/v) SDS, in order to reduce the salt and SDS concentrations, and concentrated 10-fold in a Centricon 10 before sequencing.

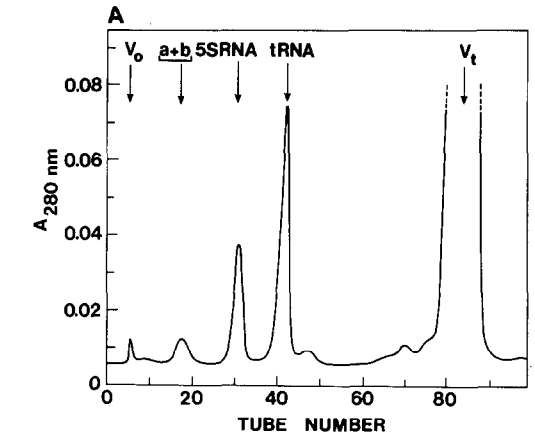
The peptides were sequenced using an Applied Biosystems 470-A gas-phase sequenator with on-line PTH analysis.

3. RESULTS AND DISCUSSION

The first step in the purification of thesaurin a from *X. laevis* immature ovaries is a sucrose density centrifugation [3,4], which separates the ribosomes (80 S) from the two thesaurisomes: the 42 S particles, described in the introduction, and the 7 S particles, made up of TFIIIA and 5 S RNA [15–17]. The fractions containing the 42 S particles were pooled, dissociated with SDS and 2-mercaptoethanol and loaded on an HPLC gel filtration preparative column (fig.1A). SDS was found to be necessary to maintain thesaurin a in solution after removal of the RNA. Three peaks were recovered between the V_o, containing high-molecular-mass aggregates and some mRNA, and the V_t peak (2-mercaptoethanol). Gel electrophoresis of the eluted fractions (fig.1B) shows that the first peak contains thesaurin a and thesaurin b and that the former protein is pure in some fractions in the leading edge of the peak (fig.1B). The second and third peaks contain 5 S RNA and tRNA, respectively.

Since the N-terminal amino acid of thesaurin a is blocked, this protein cannot be sequenced directly. Therefore, we have prepared and purified several peptides, using staphylococcal V8 protease which is active in the presence of SDS (fig.2). The peptides were separated by SDS-PAGE and recovered by electroelution with the precautions described by Hunkapiller et al. [14] to avoid N-terminal blocking. The partial sequence of the peptides a_3, a_4, a_5, a_7 and a_8 is given in fig.3 together with the corresponding sequences of *A. salina* [11] and human [12] EF-1α. There is a strong conservation of the primary structure in the sequenced regions: nearly 70% of the amino acids are identical in *X. laevis* thesaurin a and human or *A. salina* EF-1α. Furthermore, most of the differences occur in regions where human and *A. salina* EF-1α also differ (fig.3).

The question may be raised whether thesaurin a is identical to *X. laevis* EF-1α. The sequence of the *X. laevis* EF-1α (Krieg, P. and Melton, D., unpublished) demonstrates that the two proteins are different: they differ in all the positions for which a difference is noted between *X. laevis* thesaurin a and *H. sapiens* EF-1α (see fig.3). An additional difference is residue 271 which is isoleucine in *X. laevis* EF-1α. Other lines of evidences also lead to the conclusion that *X. laevis* thesaurin a is not identical to *X. laevis* EF-1α. Purified thesaurin a is highly insoluble in the absence of SDS or urea, whereas EF-1α is soluble [18]. *X. laevis* EF-1α sediments as a 5–7 S particle in the sucrose density



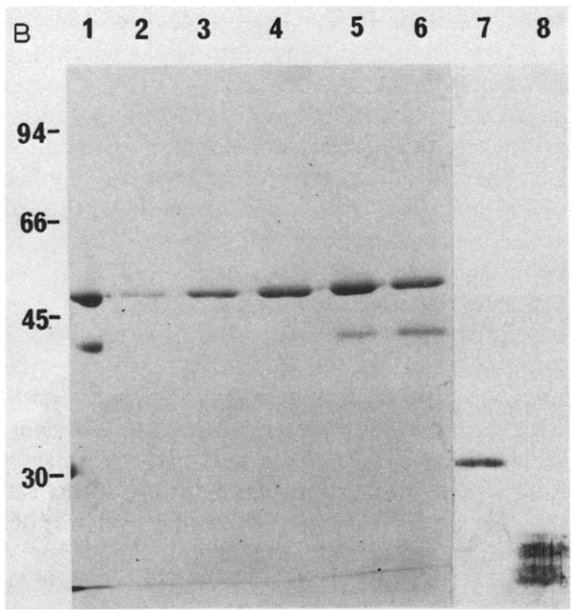


Fig.1. (A) Separation of the 42 S particle components by HPLC gel filtration chromatography. Volume of the fracations: 1.3 ml. (B) SDS-PAGE (10.4%) analysis of the HPLC fractions showing purification of thesaurin a, 5 S RNA and tRNA. Lanes: 1, 42 S particles before chromatography; 2–6, tubes 15–19 of A; 7, pool of the 5 S RNA-containing fractions; 8, pool of the tRNA-containing fractions; 1–6, Coomassie blue staining; 7 and 8, silver staining.

gradient [7], whereas thesaurin a is exclusively associated with the 42 S particles. Finally, EF-1α has a slightly higher apparent molecular mass than EF-1α on SDS-PAGE [7].

Homology between human, *A. salina* EF-1α and

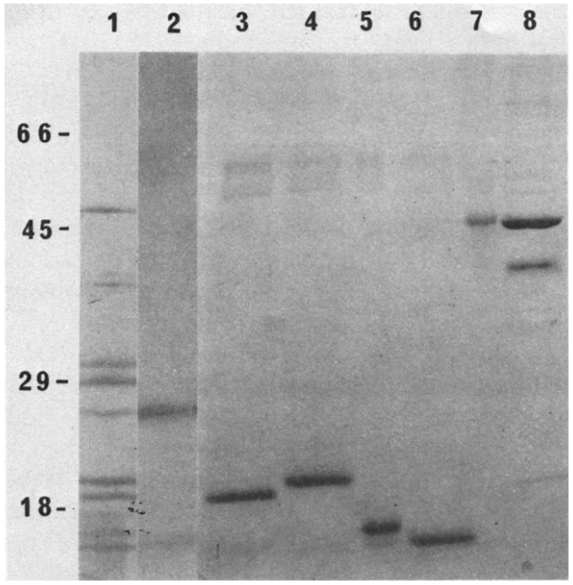


Fig.2. Purification of peptides from thesaurin a. HPLC-derived thesaurin a (lane 7) was digested by staphylococcal V8 protease (lane 1). Five peptides were purified: a_3 (lane 2), a_5 (lane 3), a_4 (lane 4), a_7 (lane 5) and a_8 (lane 6). Lane 8 contains a non-purified 42 S particle sample.

the corresponding elongation factor of bacterial and organellar origin (EF-Tu) has been demonstrated [11,12]. Since the structure of tRNA is highly conserved in evolution, it is not surprising that the sequences of the proteins whose main function is to bind aminoacyl tRNA are also highly conserved. In particular, Lys 272, one of the residues involved in tRNA binding, is conserved in thesaurin a, in all of the EF-1αs sequenced so far [12] and in EF-Tu [19]. Residues 144–149 which are considered part of a guanine nucleotide binding site (called region II by Brands et al. [12]) are identical in thesaurin a and in EF-1α, known as a GTP-binding protein. These residues are also found in other GTP-binding proteins or in the RAS proteins [12].

We speculate that thesaurin a is a translation factor modified to perform a dual function in previtellogenic oocytes: (i) transfer of aminoacyl tRNA to the ribosomes during elongation [6,7]; and (ii) long-term storage of aminoacyl tRNA [3,9]. The latter function is prominent in previtellogenic oocytes but much less so in later

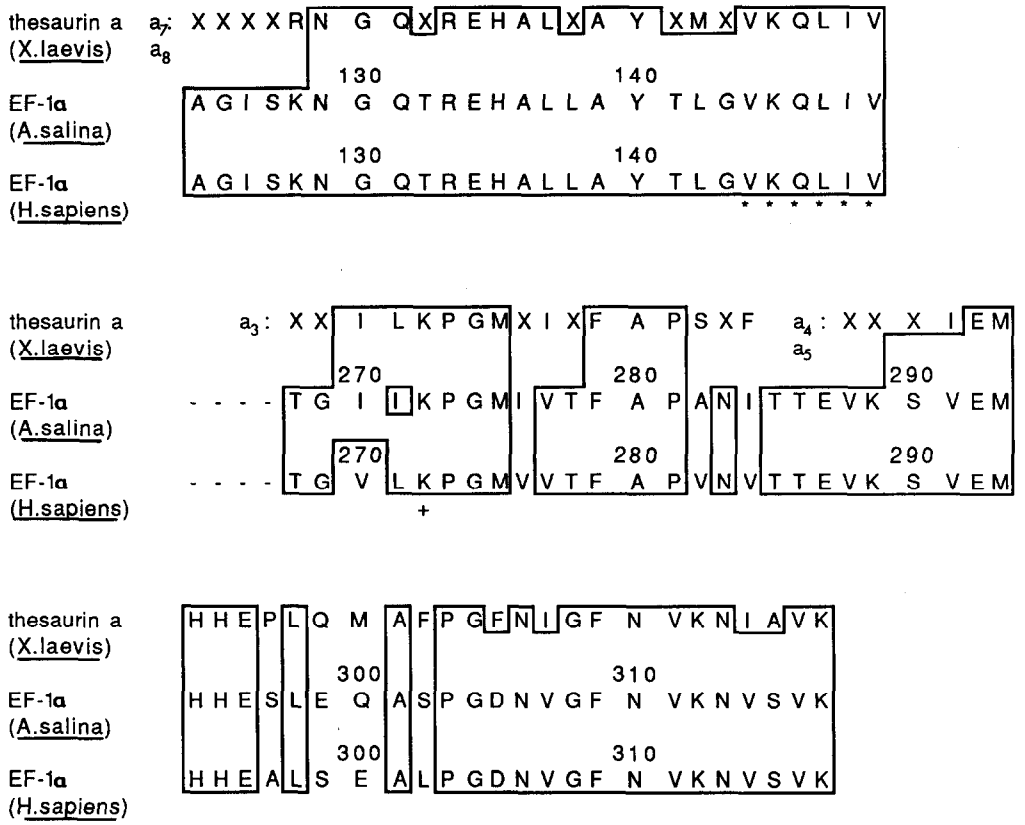


Fig.3. Comparison of the partial amino acid sequences of *X. laevis* thesaurin a, *A. salina* [11] and *H. sapiens* [12] EF-1α. Amino acid residues are shown by single letter symbols. Letter X indicates an unknown residue. Homologous sequence regions are boxed. Five V8 protease-derived peptides of thesaurin a were sequenced. Asterisks indicate the start of a guanine nucleotide binding site [12]. The plus sign (residue 272) indicates a conserved amino acid residue involved in tRNA binding [12,19]. Lysine 317 is modified as trimethyllysine in *A. salina* EF-1α [11] but not in *X. laevis* thesaurin a. Peptides a_7 and a_8 overlap partially: the first amino acid residue of peptide a_8 corresponds to the twelfth residue of peptide a_7. This is also true of peptides a_4 and a_5: the first amino acid residue of peptide a_5 corresponds to the sixth residue of peptide a_4.

oogenesis. Accordingly, thesaurin a disappears in vitellogenic oocytes [3] whereas EF-1α progressively accumulates [7]. The abundance of EF-Tu in *E. coli* (5.5% of total proteins) is such that Clark [10] has suggested a storage function for this protein. EF-1α is certainly less abundant in eukaryotic cells [20] than EF-Tu in bacteria, so that a possible storage function for EF-1α in the latest stages of oogenesis or in somatic cells would certainly be secondary to the elongation factor function. The main difference between EF-1α and thesaurin a would be that thesaurin a, not only binds aminoacyl tRNA, but is also able to interact with the other components of the 42 S particle, i.e. thesaurin b and, directly or indirectly, 5 S RNA. The resulting complex is a more efficient storage particle than the individual components in terms of bound water and hence of occupied space and also in terms of stability of the aminoacyl tRNA [9,21].

In previtellogenic oocytes, three abundant proteins are involved in 5 S RNA and tRNA storage, TFIIIA, thesaurin a and thesaurin b. It has already been shown that TFIIIA has a dual function, i.e. storage [15] and transcription of 5 S RNA [16,17]. We would not be surprised if thesaurin b, like thesaurin a and TFIIIA, also has a dual function and is involved not only in storage of RNA but also in transcription or protein synthesis.

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