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Three genes under different developmental control encode elongation factor 1-α in *Xenopus laevis*

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ABSTRACT

We have cloned cDNAs encoding two variants of the elongation factor for protein synthesis in *Xenopus laevis*, called EF-1α. One of these (42Sp50) is expressed exclusively in immature oocytes. It is one of two protein components of a 42S RNP particle that is very abundant in previtellogenic oocytes. The 42S RNP particle consists of various tRNAs, 5S RNA, 42Sp50 and a 5S RNA binding protein (42Sp43). A major function served by 42Sp50 appears to be the storage of tRNAs for later use in oogenesis and early embryogenesis. The second EF-1α variant (EF-1αO) is expressed mainly in oocytes but transiently in early embryogenesis as well. Its mRNA cannot be detected after neurulation in somatic cells. EF-1αO is closely related to a third EF-1α (EF-1αS), discovered originally by Krieg et al. (1). EF-1αS is expressed at low levels in oocytes but actively in somatic cells. The latter two proteins are very similar to known eukaryotic EF-1α from other organisms and presumably function in their respective cell types to support protein synthesis.

INTRODUCTION

Two abundant RNP storage particles ('thesaurisomes') exist in previtellogenic oocytes of anurans and teleosts (2, 3). In *Xenopus laevis* the smaller particles (7S) have a simple composition (4). They contain one molecule of 5S RNA and one molecule of the 38 kDa protein TFIIIA which has a dual function. This protein is involved in the storage of 5S RNA in immature oocytes (4). It also acts as a positive transcription factor which is required for efficient and accurate expression of 5S RNA genes (5, 6).

The larger RNP storage particle in *X. laevis* has a sedimentation coefficient of 42S and comprises four subunits each of which contains one molecule of 5S RNA, three molecules of tRNA, one molecule of a 43 kDa protein, known as 42Sp43 or thesaurin b, and two molecules of a 50 kDa protein known as 42Sp50 or thesaurin a (3). This protein has also been referred to as 42Sp48 (7). 5S RNA is associated primarily with 42Sp43 (8, 9), whereas tRNA is associated with the larger particle protein (42Sp50; 8). The interactions that hold the particle together are unknown.

The 42S particles of *X. laevis* have a metabolic activity. They can participate in protein synthesis *in vitro* by supplying the ribosomes with aminoacyl tRNA (10). After peptide bond formation, a discharged tRNA molecule is incorporated into a 42S particle, reacylated, and stored for use in another round of peptide bond formation. The tRNA in 42S particles is fully charged *in vivo* (11, 12). Purified 42S particles not only take up and reacylate tRNA (11, 12), but can directly transfer aminoacyl tRNA to ribosomes in a GTP- and mRNA-dependent reaction that mimics the well-characterized role of elongation factor EF-1α in protein synthesis (7).

More recently, 42Sp50 was found to be antigenically related to EF-1α (7), and partial amino acid sequencing of 42Sp50 confirmed this relationship (13ı). We present here the sequence of a cDNA clone encoding *X. laevis* 42Sp50. The deduced amino acid sequence of this protein is similar to that of EF-1α from other eukaryotes, confirming its identity as a member of the EF-1α family. (For a recent compilation of references to sequences of EF-1α from a variety of species, see 14). The mRNA for 42Sp50 is detected only in oocytes.

A second cDNA (EF-1αO) encoding a member of the EF-1α family has been cloned. Its mRNA is abundant in oocytes and after transient expression in early embryos progressively disappears by the end of neurulation. Its mRNA is not detectable in adult liver. Embryos and adult cells have been shown to contain mRNA encoding a third form of EF-1α that has been characterized recently by Krieg et al. (1). We propose to name this protein EF-1αS.

MATERIALS AND METHODS

Materials

Previtellogenic oocytes (stage I; 15) were obtained by digesting ovaries of immature females with collagenase (1 mg/ml in 100 mM potassium phosphate buffer, pH 7.4). Vitellogenic oocytes (stages I–V) were dissected manually from ovaries of mature

† Deceased

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                                                                  GGTTTGTTATGAAGCCTCCTCAGGTTGTCCGAGTAGGGACAAAGAAAACATCG  -241
TTTGTCAACTTCACTGATATCTGCAAACTGTTACATCGTCAGCCGAAACACTTGCTGGCCTTCTTGTTAGCTGAATTGGGGACATGTGGCTCTATAGATGGTAACAACCAGTTAGTCATC  -121
AAAGGCAGATTCCAGCAGAAGCAAATAGAGAACGTTCTAAGAAGATATATCAAGGAATACGTGACTTGTCACACCTGCCGATCTGAGTAAGTAAGTGGTTAGTAGGTTACTCGGTGCAAC    -1
ATG ACT GAC AAG GCT CCT CAA AAG ACT CAT TTG AAC ATT GTG ATT ATT GGA CAT GTT GAT TCT GGG AAA TCC ACC ACC ACC GGA CAC CTC    90
 M   T   D   K   A   P   Q   K   T   H   L   N   I   V   I   I   G   H   V   D   S   G   K   S   T   T   T   G   H   L    30
ATC TAC AAG TGC GGG GGC TTT GAC CCC AGG GCC CTG GAG AAG GTG GAG GCG GCT GCT GCT CAG CTT GGC AAG AGC TCC TTC AAG TTT GCC   180
 I   Y   K   C   G   G   F   D   P   R   A   L   E   K   V   E   A   A   A   A   Q   L   G   K   S   S   F   K   F   A    60
TGG ATC TTG GAT AAG CTG AAG GCT GAG AGG GAG CGA GGA ATC ACC ATC GAC ATC TCC CTA TGG AAG TTC CAG ACC AAC AGG TTC ACA ATC   270
 W   I   L   D   K   L   K   A   E   R   E   R   G   I   T   I   D   I   S   L   W   K   F   Q   T   N   R   F   T   I    90
ACC ATA ATC GAT GCC CCG GGG CAC AGG GAC TTC ATT AAG AAC ATG ATC ACG GGC ACC TCT CAG GCA GAT GTT GCT CTC CTG GTG GTC TCT   360
 T   I   I   D   A   P   G   H   R   D   F   I   K   N   M   I   T   G   T   S   Q   A   D   V   A   L   L   V   V   S   120
GCG GCT ACA GGG GAA TTT GAG GCC GGT GTG TCC AGA AAT GGC CAA ACA AGG GAA CAC GCT CTT CTG GCC TAC ACC ATG GGG GTC AAG CAA   450
 A   A   T   G   E   F   E   A   G   V   S   R   N   G   Q   T   R   E   H   A   L   L   A   Y   T   M   G   V   K   Q   150
CTG ATC GTC TGC GTG AAC AAA ATG GAT CTG ACG GAC CCT CCC TAC AGC CAC AAG CGG TTT GAT GAA GTT GTC AGG AAT GTG ATG GTC TAT   540
 L   I   V   C   V   N   K   M   D   L   T   D   P   P   Y   S   H   K   R   F   D   E   V   V   R   N   V   M   V   Y   180
CTG AAA AAG ATT GGG TAC AAC CCG GCT ACC ATC CCC TTC GTG CCT GTG TCT GGC TGG ACG GGA GAG AAT ATA TCT TCG CCC AGT CAA AAG   630
 L   K   K   I   G   Y   N   P   A   T   I   P   F   V   P   V   S   G   W   T   G   E   N   I   S   S   P   S   Q   K   210
ATG GGT TGG TTT AAA GGT TGG AAG GTG AAA CGA AAA GAT GGC TTT ACA AAG GGC CAA TCC CTC TTG GAG GTT CTG GAT GCG CTT GTA CCT   720
 M   G   W   F   K   G   W   K   V   K   R   K   D   G   F   T   K   G   Q   S   L   L   E   V   L   D   A   L   V   P   240
CCA GTG AGG CCG GCA AAT AAG CCT TTG CGG CTC CCC CCT GCA TAT GTA TAC AAG ATA GGG GGC ATT GGT ACA GTC CCT GTG GGC AAG ATA   810
 P   V   R   P   A   N   K   P   L   R   L   P   P   A   Y   V   Y   K   I   G   G   I   G   T   V   P   V   G   K   I   270
GAA ACT GGG ATT CTG AAG CCA GGC ATG ACC ATC TCC TTT GCA CCG TCT GGT TTC TCA GCT GAA GTT AAA TCC ATA GAA ATG CAC CAC GAG   900
 E   T   G   I   L   K   P   G   M   T   I   S   F   A   P   S   G   F   S   A   E   V   K   S   I   E   M   H   H   E   300
CCG CTT CAG ATG GCC TTC CCA GGG TTC AAC ATC GGA TTC AAT GTC AAG AAC ATT GCT GCG AAA AGT CTA AAG CGT GGC AAT GTG GCG GGC   990
 P   L   Q   M   A   F   P   G   F   N   I   G   F   N   V   K   N   I   A   A   K   S   L   K   R   G   N   V   A   G   330
AAT TCA AAG AGT GAC CCA CCG ACT GAG GCC TCC AGC TTC ACT GCC CAG GTG ATC ATT CTG AAC CAC CCG GGC TTT ATC AAA GCC GGA TAT  1080
 N   S   K   S   D   P   P   T   E   A   S   S   F   T   A   Q   V   I   I   L   N   H   P   G   F   I   K   A   G   Y   360
TCA CCG GTT ATC GAC TGT CAC ACT GCA CAC ATC ACA TGC CAG TTT GCA GAA CTG CAG GAA AAG ATT GAC AGG CGG ACT GGC AAA AAG CTA  1170
 S   P   V   I   D   C   H   T   A   H   I   T   C   Q   F   A   E   L   Q   E   K   I   D   R   R   T   G   K   K   L   390
GAG GAC AAC CCG GGG CTA CTG AAA TCT GGA GAT GCC GCC ATC ATA ACC CTG AAG CCC ATC AAG CCC TTC TGT GTG GAG AGG TTC TTT GAT  1260
 E   D   N   P   G   L   L   K   S   G   D   A   A   I   I   T   L   K   P   I   K   P   F   C   V   E   R   F   F   D   420
TAT CCA CCT CTA GGG AGG TTT GCA GCC CGA GAC CTA AAA CAG ACT GTT GCC GTC GGG GTT GTG AAG TCG GTG GAG CAC AAA GCT GGA GCT  1350
 Y   P   P   L   G   R   F   A   A   R   D   L   K   Q   T   V   A   V   G   V   V   K   S   V   E   H   K   A   G   A   450
GCT GCC AGG AGA CAA GTC CAA AAA CCA GTG TTG GTG AAG TGA CTTTATGCAGAATTGGAGACGTAAAGAAAGTGCTTAGGCCGGAGCAGGCACTAATTTATTTTT  1455
 A   A   R   R   Q   V   Q   K   P   V   L   V   K  ***
TGTTTGGTTGTGACAGTTTTACATGACAGTGAAAAGGAAACTGTTGTGTGTAAAGTAAATAAACTGATTGTGCAAGTAACGGCATTTGTCAGTCTTCTCTGAACAGGAGATTCTATAGGG  1575
GGTTTGGGTAATGGACATAAGATTCCTCTGTGTATAGACTCTGCTTTCCCCTGTTTCAGCTCAGTTACAACTGGTATAAAAATAAAAACTGGGTCAATAGCTGTGCCAAATAAAAGATGC  1695
ATCAAATATAATTAGGCAAAAATGTAATGTATAAAGCAGGGATCCCCAACCTCTTGAACCTGTGAGCAACATTCAGAAGTAACGAGTTGTGGAGCAACACTAGCATGAAAAATATTCTTG  1815
GGGTGACAAGTGCTGTCATTGGCCAGTCAACCTACATTTAGGCTCTGTATGGCACTGCACCTGGTTTTTATACAACCAAAACTTCTAAGTCA                              1907
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Figure 1. Nucleotide sequence of the cDNA clone Xl42Sp50 and deduced amino acid sequence of 42Sp50 (thesaurin a) protein. The cDNA sequence contains 293 bases of 5′ untranslated sequence, 1389 bases of coding sequence and 518 bases of 3′ untranslated sequence. The latter sequence contains 3 putative polyadenylation signals (underlined), but no poly(A) tract. The sequence of 3 peptides derived from 42Sp50 protein (13) is overlined.

females. The cells were rinsed in Barth's medium (16), collected in batches of 50–100 and frozen in liquid nitrogen. Embryos were staged according to the normal table of Nieuwkoop and Faber (17) and immediately processed for RNA purification.

Purification of RNA and analysis of transcripts

Total RNA was purified from liver, ovaries, oocytes and embryos by the LiCl-urea method (18). Poly-A$^+$ RNA was prepared by oligodT chromatography according to standard procedures (19). An amount of RNA that corresponds to the content of 5–10 oocytes or embryos was used for Northern blots.

Aliquots of total or poly(A)$^+$ RNA were fractionated in 1% agarose gels containing formaldehyde (19), transferred to nitrocellulose membranes or nylon, and hybridized with the ^{32}P-labeled insert of various cDNA clones. Hybridization was carried out overnight at 42°C in 5×SSPE, 50% formamide (19). The membranes were washed at 42°C in 0.1×SSPE, dried and autoradiographed.

Screening of cDNA libraries

About 10^6 clones from an oocyte cDNA library in lambda gt10 (20) were screened with a *X. laevis* EF-1α cDNA probe (1). A dozen positive clones were recovered. These clones could be classified in three groups according to the intensity of the hybridization signals with EF-1α cDNA. Two clones (Xl7 and Xl8) giving a signal of intermediate intensity were selected and analyzed further. Both of them contained an insert of about 1300 bp which was similar in sequence (85% identical residues) with the 3′ part of EF-1αS cDNA (1). The 5′ part of clone Xl8 was subcloned and used as a probe to search for longer cDNA clones in the lambda gt10 library. Two clones were obtained with inserts of 1493 and 1424 bp, respectively. The 1493 bp clone (Xl9) is a longer version of Xl7 and Xl8. It is described here as EF-1αO. The 1424 bp clone (Xl10) is slightly different in sequence from EF-1αO. The Xl9 (EF-1αO) and Xl10 (EF-1αO1) cDNAs might be due to polymorphism of a single gene or derived from two different genes.

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                                                                      CGGCAATTGTCTAGCCGTCGCTGCCAAG    -1
ATG GGA AAG GAA AAG ATT CAC ATT AAC ATC GTC GTG ATC GGG CAT GTG GAC TCG GGC AAG TCA ACC ACC ACC GGG CAT CTC ATC TAC AAA    90
 M   G   K   E   K   I   H   I   N   I   V   V   I   G   H   V   D   S   G   K   S   T   T   T   G   H   L   I   Y   K    30
TGC GGG GGC ATC GAC AAA AGA ACC ATC GAG AAG TTT GAG AAG GAA GCT GCG GAG ATG GGT AAA GGC TCC TTT AAA TAT GCT TGG GTC TTG   180
 C   G   G   I   D   K   R   T   I   E   K   F   E   K   E   A   A   E   M   G   K   G   S   F   K   Y   A   W   V   L    60
GAT AAG TTG AAA GCT GAG CGA GAA CGT GGA ATA ACA ATC GAT ATT TCC TTG TGG AAG TTT GAG ACT GGA AAA TTC TAT ATA ACT ATT ATT   270
 D   K   L   K   A   E   R   E   R   G   I   T   I   D   I   S   L   W   K   F   E   T   G   K   F   Y   I   T   I   I    90
GAT GCT CCT GGC CAC AGA GAC TTT ATC AAA AAC ATG ATC ACT GGC ACT TCT CAG GCT GAC TGT GCT GTG CTC ATT GTT GCT GGT GGT GTT   360
 D   A   P   G   H   R   D   F   I   K   N   M   I   T   G   T   S   Q   A   D   C   A   V   L   I   V   A   G   G   V   120
GGC GAA TTT GAG GCT GGT ATC TCT AAA AAT GGA CAG ACT CGG GAG CAT GCC CTC TTA GCT TTC ACC CTA GGT GTC AAG CAG CTT ATA ATT   450
 G   E   F   E   A   G   I   S   K   N   G   Q   T   R   E   H   A   L   L   A   F   T   L   G   V   K   Q   L   I   I   150
GGA GTT AAC AAG ATG GAC TCC ACT GAG CCC CCT TTT AGC CAA AAA AGG TTT GAA GAA ATT ACT AAA GAA GTC AGT GCC TAC ATT AAG AAG   540
 G   V   N   K   M   D   S   T   E   P   P   F   S   Q   K   R   F   E   E   I   T   K   E   V   S   A   Y   I   K   K   180
ATT GGC TAC AAC CCA GCG ACT GTT CCA TTT GTG CCA ATA TCT GGA TGG CAT GGA GAC AAC ATG CTG GAG GCT AGC ACC AAT ATG CCC TGG   630
 I   G   Y   N   P   A   T   V   P   F   V   P   I   S   G   W   H   G   D   N   M   L   E   A   S   T   N   M   P   W   210
TTT AAA GGC TGG AAG ATT GAA AGG AAA GAG GGA AAT GCC AGT GGT GTA ACT CTG CTA GAA GCA CTT GAC TGT ATT ATT CCT CCT CAG AGG   720
 F   K   G   W   K   I   E   R   K   E   G   N   A   S   G   V   T   L   L   E   A   L   D   C   I   I   P   P   Q   R   240
CCC ACT GCC AAG CCT CTA CGG CTC CCT CTG CAG GAT GTG TAC AAG ATT GGT GGA ATT GGT ACC GTG CCT GTT GGC AGA GTG GAG ACT GGT   810
 P   T   A   K   P   L   R   L   P   L   Q   D   V   Y   K   I   G   G   I   G   T   V   P   V   G   R   V   E   T   G   270
GTC TTA AAG CCT GGT ATG ATT GTG ACT TTT GCA CCA AGT AAT GTC ACT ACA GAA GTA AAG TCT GTG GAA ATG CAT CAT GAG GCT TTG CAA   900
 V   L   K   P   G   M   I   V   T   F   A   P   S   N   V   T   T   E   V   K   S   V   E   M   H   H   E   A   L   Q   300
GAG GCA TTG CCT GGA GAC AAT GTT GGT TTC AAT GTG AAG AAC ATA TCT GTA AAG GAC ATT AGG AGA GGC AAC GTA GCT GGT GAC AGC AAA   990
 E   A   L   P   G   D   N   V   G   F   N   V   K   N   I   S   V   K   D   I   R   R   G   N   V   A   G   D   S   K   330
AAT GAC CCA CCT ATG CAA GCT GGC AGT TTT ACC GCA CAG GTG ATC ATA CTC AAT CAC CCA GGA CAG ATT AGT GCT GGT TAT GCT CCA GTC  1080
 N   D   P   P   M   Q   A   G   S   F   T   A   Q   V   I   I   L   N   H   P   G   Q   I   S   A   G   Y   A   P   V   360
CTG GAC TGT CAC ACA GCT CAT ATT GCT TGT AAG TTT GCT GAG CTG AAG CAA AAG ATT GAC CGA AGA AGT GGC AAG AAG CTG GAA GAT GAC  1170
 L   D   C   H   T   A   H   I   A   C   K   F   A   E   L   K   Q   K   I   D   R   R   S   G   K   K   L   E   D   D   390
CCA AAA TTC TTG AAA TCT GGA GAT GCA GCT ATA GTG GAA ATG ATT CCT GGG AAG CCC ATG TGT GTA GAA AGC TTT TCT GAC TAT CCT CCA  1260
 P   K   F   L   K   S   G   D   A   A   I   V   E   M   I   P   G   K   P   M   C   V   E   S   F   S   D   Y   P   P   420
CTT GGC CGA TTT GCA GTT CGT GAT ATG AGG CAG ACG GTG GCT GTT GGA GTA ATC AAA GGT GTG GAC AAA AAG GCA GCA AGT TCT GGG AAA  1350
 L   G   R   F   A   V   R   D   M   R   Q   T   V   A   V   G   V   I   K   G   V   D   K   K   A   A   S   S   G   K   450
GTA ACA AAG TCT GCT GTG AAA GCT GGA AAG AAA TGA TTGATGTACAGTTTAATCTTGGTGTCCTGTAAACTTAATAAATGTTTAATGATGCTTTTGAAAAAAAAAAA  1457
 V   T   K   S   A   V   K   A   G   K   K  ***
AAAAAAAA                                                                                                                  1465
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Figure 2. Nucleotide sequence of cDNA clone X19 and derived amino acid sequence of EF-1αO protein. The clone contains 28 bases of 5′ untranslated sequence, 1383 bases of coding sequence and 63 bases of 3′ untranslated sequence. A polyadenylation signal (underlined) lies 18 bases upstream of a 19 base poly(A) tract.

```
                  10        20        30        40        50        60        70        80        90       100       110       120
eEF-1αS   MGKEKTHIKIVVIGHVDSGKSTTTGHLIYKCGGIDKRTIEKFEKEAAEMGKGSFKYAWVLDKLKAERERGITIDISLWKFETSKYYVTIIDAPGHRDFIKNMITGTSQADCAVLIVAAGV
eEF-1αO   MGKEKIHINIVVIGHVDSGKSTTTGHLIYKCGGIDKRTIEKFEKEAAEMGKGSFKYAWVLDKLKAERERGITIDISLWKFETGKFYITIIDAPGHRDFIKNMITGTSQADCAVLIVAGGV
42Sp50 MTDKAPQKTHLNIVIIGHVDSGKSTTTGHLIYKCGGFDPRALEKVEAAAAQLGKSSFKFAWILDKLKAERERGITIDISLWKFQTNRFTITIIDAPGHRDFIKNMITGTSQADVALLVVSAAT

                 130       140       150       160       170       180       190       200       210       220       230       240
          GEFEAGISKNGQTREHALLAYTLGVKQLIVGINKMDSTEPPYSQKRYEEIVKEVSTYIKKIGYNPDTVAFVPISGWNGDNMLEPSPNMPWFKGWKITRKEGSGSGTTLLEALDCILPPSR
          GEFEAGISKNGQTREHALLAFTLGVKQLIIGVNKMDSTEPPFSQKRFEEITKEVSAYIKKIGYNPATVPFVPISGWHGDNMLEASTNMPWFKGWKIERKEGNASGVTLLEALDCIIPPQR
          GEFEAGVSRNGQTREHALLAYTMGVKQLIVCVNKMDLTDPPYSHKRFDEVVRNVMVYLKKIGYNPATIPFVPVSGWTGENISSPSQKMGWFKGWKVKRKDGFTKGQSLLEVLDALVPPVR

                 250       260       270       280       290       300       310       320       330       340       350       360
          PTDKPLRLPLQDVYKIGGIGTVPVGRVETGVIKPGMVVTFAPVNVTTEVKSVEMHHEALTEAVPGDNVGFNVKNVSVKDVRRGNVAGDSKNDPPMEAGSFTAQVIILNHPGQIGAGYAPV
          PTAKPLRLPLQDVYKIGGIGTVPVGRVETGVLKPGMIVTFAPSNVTTEVKSVEMHHEALQEALPGDNVGFNVKNISVKDIRRGNVAGDSKNDPPMQAGSFTAQVIILNHPGQISAGYAPV
          PANKPLRLPPAYVYKIGGIGTVPVGKIETGILKPGMTISFAPSGFSAEVKSIEMHHEPLQMAFPGFNIGFNVKNIAAKSLKRGNVAGNSKSDPPTEASSFTAQVIILNHPGFIKAGYSPV

                 370       380       390       400       410       420       430       440       450       460
          LDCHTAHIACKFAELKEKIDRRSGKKLEDNPKFLKSGDAAIVDMIPGKPMCVESFSDYPPLGRFAVRDMRQTVAVGVIKAVEKKAAGSGKVTKSAQKAAKTK
          LDCHTAHIACKFAELKQKIDRRSGKKLEDDPKFLKSGDAAIVEMIPGKPMCVESFSDYPPLGRFAVRDMRQTVAVGVIKGVDKKAASSGKVTKSAVKAGK-K
          IDCHTAHITCQFAELQEKIDRRTGKKLEDNPGLLKSGDAAIITLKPIKPFCVERFFDYPPLGRFAARDLKQTVAVGVVKSVEHKAGAAAR--RQVQKPVLVK
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Figure 3. Alignment of amino acid sequences of EF-1αS (1), EF-1αO (Fig. 2) and 42Sp50 (Fig. 1). Colons indicate identical residues. Points indicate conservative substitutions (22).

The clone encoding 42Sp50 was identified from a lambda gt11 expression library by means of a polyclonal antibody prepared against gel purified 42Sp50.

DNA sequencing

DNA sequences were determined for both strands by the chain termination method of Sanger et al. (21). Overlapping single-

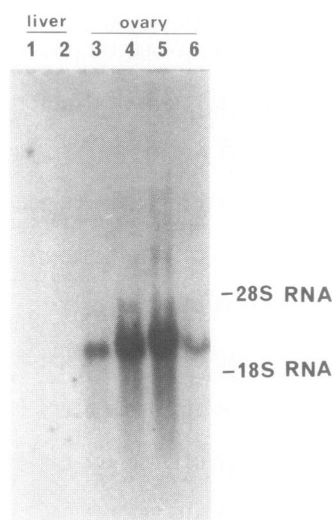

Figure 4. RNA blot analysis of 42Sp50 expression in liver and ovary. Lane 1, 2 μg of poly(A)$^+$ RNA from liver; lane 2, 64 μg of total RNA from liver; lane 3, 2 μg of poly(A)$^+$ RNA from immature ovaries (including only stage I oocytes); lane 4, 9 μg of total RNA from immature ovaries (inclucing only stage I oocytes); lane 5, 22 μg of total RNA from immature ovaries (including stages I and II oocytes); lane 6, 22 μg of total RNA from mature ovaries (including stages I to VI oocytes). The RNA was fractionated on a 1% agarose gel containing formaldehyde, transferred to a nitrocellulose membrane and hybridized with a labeled 42Sp50 cDNA probe. After washing and drying, the filter was exposed to an X-ray film for 24 hr. The position of 28S and 18S RNA (4000 and 1800 bases, respectively) is indicated.

stranded fragments of the original cDNA inserts were generated as described in the Cyclone kit (IBI), and sequenced by extension of M13 standard or reverse primers.

RESULTS

Cloning and sequencing of 42Sp50 and EF-1αO

The cDNA encoding 42Sp50 was isolated from a lambda gt11 expression library prepared from ovary mRNA. Polyclonal antibody was prepared from gel purified p50. The clone can be identified unequivocally as encoding 42Sp50 since it contains the sequences of three polypeptides derived from 42Sp50. (13) (Fig. 1).

The cDNA encoding EF-1αO was identified from a lambda gt10 library by its hybridization with the cDNA of EF-1αS. Its sequence is shown in Fig. 2. Sequences of the three different EF-1α proteins, deduced from their cDNA sequence, are compared in Fig. 3. The somatic and oocyte EF-1α (EF-1αO and EF-1αS) are closely related with 91% of their residues identical, while the RNA storage particle protein 42Sp50 is much more diverged having only 69% identical residues. The extreme N and C termini of 42Sp50 are completely different from the two other proteins.

Expression of the three EF-1α genes during development

We compared the expression of the three genes in oogenesis and throughout embryonic development by Northern blots. The mRNA encoding 42Sp50 is most abundant in immature oocytes

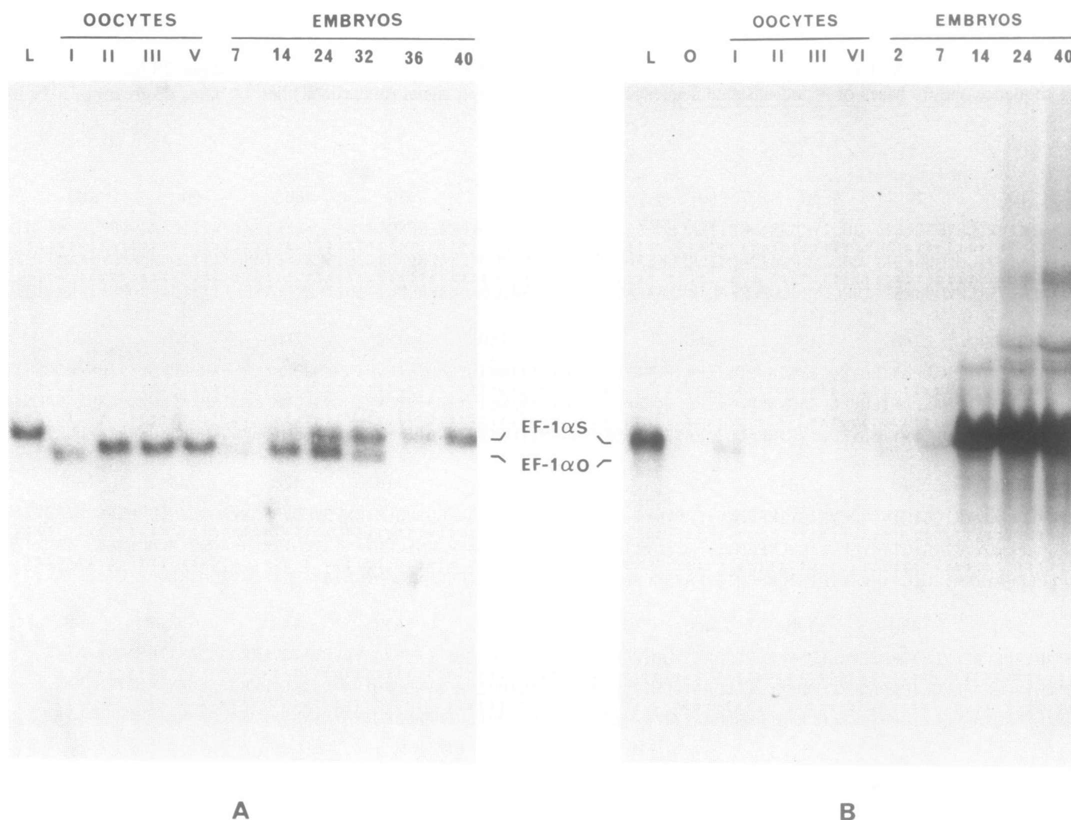

Figure 5. RNA blot analysis of EF-1αO (A) and EF-1αS (B) expression in oocytes and embryos. All lanes contain total RNA from 10 oocytes or 5 embryos, except those labeled L and O which contain 0.4 μg of poly(A)$^+$ RNA from liver and from mature ovaries, respectively. The position of EF-1αO and EF-1αS mRNA is indicated. The autoradiograms presented are 20 hr (A) and 8 hr (B) exposures.

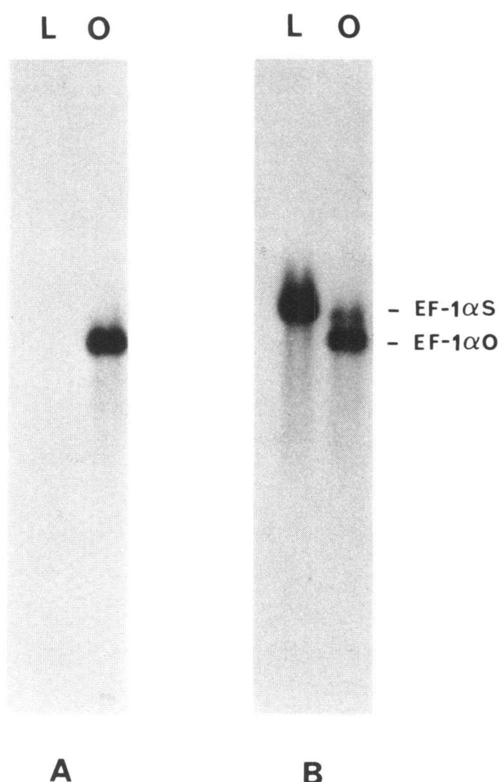

Figure 6. RNA blot analysis of EF-1αO and EF-1αS expression in liver and ovary. Lane L, 22 μg of total RNA from liver; lane O, 2 μg of poly(A)$^+$ RNA from immature ovaries (including only stage I oocytes). The RNA was fractionated on a 1% agarose gel as in Fig. 4, transferred to a nitrocellulose membrane and hybridized with a labeled EF-1αO probe (A). After a 24 hr exposure to an X-ray film, the filter was hybridized under the same conditions with an EF-αS probe and autoradiographed for another 24 hr (B).

containing stage I and stage II oocytes, detectable in mature oocytes, but undetectable in liver (Fig. 4). Experiments (not shown) demonstrate that the mRNA for 42Sp50 is present only in stage I oocytes. The mRNA for EF-1αO accumulates during oogenesis, is transiently expressed after the mid blastula transition, but then disappears after the end of neurulation (Fig. 5A). Adult liver has no detectable mRNA for EF-1αO (Fig. 6). The third member of the EF-1α family is EF-1αS. Its mRNA migrates more slowly than that of EF-1αO so that mixtures of the two can be distinguished. We confirm the results of Krieg et al. (1) who demonstrated that this gene is expressed very actively at the mid blastula transition of embryogenesis and continues to be expressed in somatic cells (Fig. 5B). We also detect small amounts of this mRNA in oocytes. Thus, it is clear that these three related genes are regulated very differently in development.

DISCUSSION

By virtue of their similarity to the other known EF-1α proteins sequenced from a variety of eukaryotes, we conclude that EF-1αO and EF-1αS perform the well-known functions of transferring aminoacyl tRNA to the ribosome, but presumably in different cell types. These two closely related *Xenopus* proteins are 75–90% similar in their amino acid sequence to EF-1α's from mammalian and other eukaryotic sources. The more distantly related protein 42Sp50 shares the ability to bind a variety of different tRNAs (7). However, this protein has the novel property of interacting with the other components of the 42S RNP particle that is unique to oocytes (3). At least one function of this particle is the storage of 5S RNA and various tRNAs (23). Presumably, the divergence of 42Sp50 from the more traditional and highly conserved forms of EF-1α reflects, in part, this different oocyte-specific function.

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