# Two Forms of Elongation Factor 1α (EF-1α O and 42Sp50), Present in Oocytes, but Absent in Somatic Cells of *Xenopus laevis*

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***Abstract.*** We have purified and partially sequenced the EF-1α protein from *Xenopus laevis* oocytes (EF-1αO). We show that the two cDNA clones isolated by Coppard et al. (Coppard, N. J., K. Poulsen, H. O. Madsen, J. Frydenberg, and B. F. C. Clark. 1991. *J. Cell Biol.* 112:237–243) do not encode 42Sp50, as claimed by these authors, but two very similar forms of EF-1αO (EF-1αO and EF-1αO1). 42Sp50 is the major protein component of a 42S nucleoprotein particle that is very abundant in previtellogenic oocytes of *X. laevis*. 42Sp50 differs from EF-1αO not only by its amino acid sequence, but also by several properties already reported. In particular, 42Sp50 has a low EF-1α activity. It is distributed uniformly in the cytoplasm of previtellogenic oocytes, in contrast to EF-1αO which is concentrated in a small region of the cytoplasm, known as the mitochondrial mass or Balbiani body.

SEVERAL genes encode elongation factor 1α (EF-1α) in *Xenopus laevis* (Djé et al., 1990). The known members of this gene family are EF-1αS, EF-1αO, and 42Sp50.[1] EF-1αS is the somatic form of EF-1α. This form is expressed in embryos beginning at the mid-blastula transition, and in adult cells (Krieg et al., 1989; Djé et al., 1990). EF-1αO and 42Sp50 are oocyte-specific forms of EF-1α. These proteins differ by several properties. First, the tRNA transfer activity of EF-1αO is at least one order of magnitude higher than that of 42Sp50 (Mattaj et al., 1987; Viel et al., 1991). Second, EF-1αO is not a component of the 42S nucleoprotein particles, as is 42Sp50 (Picard et al., 1980; Viel et al., 1990). Third, EF-1αO is present in oocytes of all stages, whereas 42Sp50 is present only in previtellogenic oocytes (Viel et al., 1990). Fourth, EF-1αO is concentrated in the mitochondrial mass of previtellogenic oocytes (Viel et al., 1990), whereas 42Sp50 is distributed uniformly in the cell cytoplasm (Mattaj et al., 1983; Viel et al., 1990).

In a recent paper Coppard et al. (1991) reported the isolation of two closely related cDNA clones (42Sp48-1 and 42Sp48-2), which they claim encode 42Sp50. In fact, these clones do not encode 42Sp50 but two very similar forms of EF-1αO, described by us as EF-1αO and EF-1αO1 (Djé et al., 1990; these sequence data are available from EMBL/Gen Bank DDBJ under accession numbers X52976 and X52977). Another cDNA clone, also isolated by us encodes 42Sp50 (Djé et al., 1990; these sequence data are available from EMBL/GenBank/DDBJ under accession number X52975).

To avoid confusion, we present below evidence establishing the correct identity of the clones belonging to the EF-1α family. We have purified and partially sequenced EF-1α from *X. laevis* ovaries. The amino acid sequence of this protein corresponds to that of EF-1αO cDNA (Djé et al., 1990). It differs markedly from that encoded by 42Sp50 cDNA (Djé et al., 1990) and from that of 42Sp50 protein (Viel et al., 1987).

## Materials and Methods

EF-1α was purified from ovaries of mature females by the procedure of Miyazaki et al. (1988). Full details of the purification procedure will be given elsewhere. 42S particles and 42Sp50 were purified from immature ovaries as described previously (Viel et al., 1991).

The purity of the particle and protein preparations was checked by polyacrylamide gel electrophoresis (Laemmli, 1970) and immunoblotting using antibodies raised against *Artemia salina* EF-1α or *X. laevis* 42S particle proteins (Viel et al., 1990).

Purified EF-1α (120 μg/ml) was dialyzed overnight at room temperature against 500 ml of V8 buffer (125 mM Tris-HCl, pH 6.8, 1 mM EDTA, 0.1% SDS), concentrated in Centricon 10 (Amicon Corp., Danvers, MA), and digested for 1 h at 37°C with staphylococcal V8 protease (Miles Laboratories Inc., Naperville, IL) at a protein/enzyme ratio of 10:1 (wt/wt). Under these conditions, protease V8 cuts the peptide bonds between glutamate and various amino acids, except proline (Deschamps et al., 1991). Proteolysis was stopped by boiling the digest in 10% 2-mercaptoethanol, 2% SDS. The resulting peptides were separated by SDS-gel electrophoresis, transferred to a polyvinylidene difluoride membrane (Matsudaira, 1987), and stained with Coomassie blue. A strip containing the peptide band was cut from the membrane and submitted to Edman degradation in a gas-phase sequenator

1. In previous publications (Viel et al., 1987, 1990, 1991) 42Sp50 is also called thesaurin a.

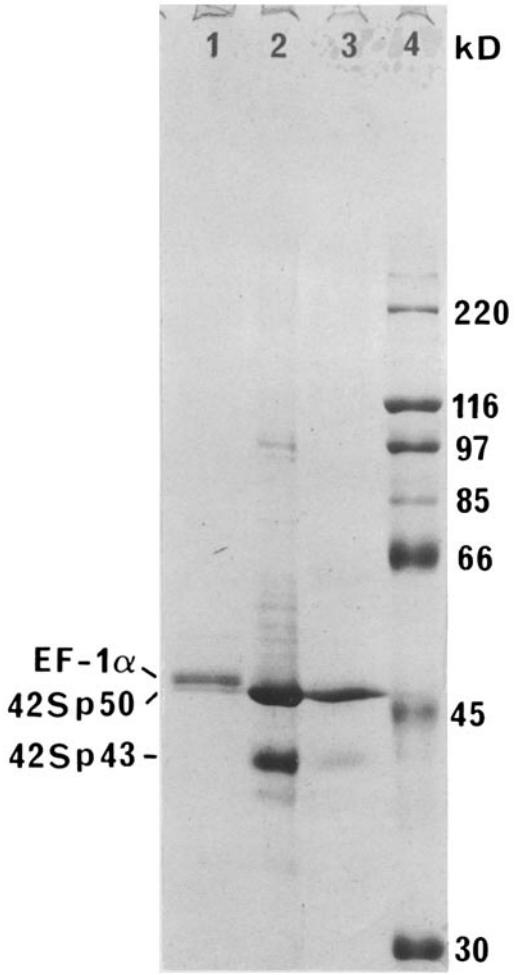


*Figure 1.* Electrophoretic analysis of EF-1α purified from mature ovaries. Lane *1* contains 0.5 μg of purified EF-1α. Lane *2* contains 3.3 μg of 42S particle proteins (42Sp50 and 42Sp43). Lane *3* contains 1.4 μg of purified 42Sp50. Lane *4* contains protein markers of known sizes. EF-1α (lane *1*) is slightly contaminated with 42Sp50 contributed by previtellogenic oocytes present in the ovaries.

(model 170-A; Applied Biosystems Inc., Foster City, CA) coupled to a PTH analyzer.

## *Results and Discussion*

EF-1α purified from ovaries has a slightly lower electrophoretic mobility than 42Sp50 (Fig. 1). This confirms previous observations (Mattaj et al., 1987; Viel et al., 1991). The major band seen in Fig. 1 strongly reacts with an EF-1α antiserum, whereas the minor band reacts with a 42Sp50 antiserum (data not shown). The latter band corresponds to 42Sp50 contributed by previtellogenic oocytes present in mature ovaries. When EF-1α is purified from defolliculated vitellogenic oocytes no contamination with 42Sp50 can be detected (data not shown).

Several peptides were generated from oocyte EF-1α by digestion with protease V8, purified by gel electrophoresis, and sequenced by Edman degradation. Four short stretches of sequence were elucidated, including a total of 23 residues (Fig. 2, line *1*). They perfectly match the amino acid sequence encoded by EF-1αO cDNA (Djé et al., 1990) and

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1. EF-1αO protein                                                  EXAEMG  50
2. EF-1αO cDNA    MGKEKIHINI VVIGHVDSGK STTTGHLIYK CGGIDKRTIE KFEKEAAEMG
3. EF-1αS cDNA    MGKEKTHINI VVIGHVDSGK STTTGHLIYK CGGIDKRTIE KFEKEAAEMG
4. 42Sp50 cDNA MTDKAPQKTHLNI VIIGHVDSGK STTTGHLIYK CGGFDPRALE KVEAAAAQLG

2. EF-1αO cDNA    KGSFKYAWVL DKLKAERERG ITIDISLWKF ETGKFYITII DAPGHRDFIK 100
3. EF-1αS cDNA    KGSFKYAWVL DKLKAERERG ITIDISLWKF ETSKYYVTII DAPGHRDFIK
4. 42Sp50 cDNA    KSSFKFAWIL DKLKAERERG ITIDISLWKF QTNRFTITII DAPGHRDFIK

2. EF-1αO cDNA    NMITGTSQAD CAVLIVAGGV GEFEAGISKN GQTREHALLA FTLGVKQLII 150
3. EF-1αS cDNA    NMITGTSQAD CAVLIVAAGV GEFEAGISKN GQTREHALLA YTLGVKQLIV
4. 42Sp50 cDNA    NMITGTSQAD VALLVVSAAT GEFEAGVSRN GQTREHALLA YTMGVKQLIV
5. 42Sp50 protein                          EXXXXRN GQXREHALXA YXMXVKQLIV
                                           o                  o o      o

2. EF-1αO cDNA    GVNKMDSTEP PFSQKRFEEI TKEVSAYIKK IGYNPATVPF VPISGWHGDN 200
3. EF-1αS cDNA    GINKMDSTEP PYSQKRYEEI VKEVSTYIKK IGYNPDTVAF VPISGWNGDN
4. 42Sp50 cDNA    CVNKMDLTDP PYSHKRFDEV VRNVMVYLKK IGYNPATIPF VPVSGWTGEN

2. EF-1αO cDNA    MLEASTNMPW FKGWKIERKE GNASGVTLLE ALDCIIPPQR PTAKPLRLPL 250
3. EF-1αS cDNA    MLEPSPNMPW FKGWKITRKE GSGSGTTLLE ALDCILPPSR PTDKPLRLPL
4. 42Sp50 cDNA    ISSPSQKMGW FKGWKVKRKD GFTKGQSLLE VLDALVPPVR PANKPLRLPP

1. EF-1αO protein                  EXX VL                EVK SVEM  EALQ 300
2. EF-1αO cDNA    QDVYKIGGIG TVPVGRVETG VLKPGMIVTF APSNVTTEVK SVEMHHEALQ
3. EF-1αS cDNA    QDVYKIGGIG TVPVGRVETG VIKPGMVVTF APVNVTTEVK SVEMHHEALT
4. 42Sp50 cDNA    AYVYKIGGIG TVPVGKIETG ILKPGMTISF APSGFSAEVK SIEMHHEPLQ
5. 42Sp50 protein                  EXX ILKPGMXIXF APSXF  EXX XIEMHHEPLQ
                                   o       o       o      o   o

1. EF-1αO protein EALP
2. EF-1αO cDNA    EALPGDNVGF NVKNISVKDI RRGNVAGDSK NDPPMQAGSF TAQVIILNHP 350
3. EF-1αS cDNA    EAVPGDNVGF NVKNVSVKDV RRGNVAGDSK NDPPMEAGSF TAQVIILNHP
4. 42Sp50 cDNA    MAFPGFNIGF NVKNIAAKSL KRGNVAGNSK SDPPTEASSF TAQVIILNHP
5. 42Sp50 protein MAFPGFNIGF NVKNIAVK
                  o o o o       o+

2. EF-1αO cDNA    GQISAGYAPV LDCHTAHIAC KFAELKQKID RRSGKKLEDD PKFLKSGDAA 400
3. EF-1αS cDNA    GQIGAGYAPV LDCHTAHIAC KFAELKEKID RRSGKKLEDN PKFLKSGDAA
4. 42Sp50 cDNA    GFIKAGYSPV IDCHTAHITC QFAELQEKID RRTGKKLEDN PGLLKSGDAA

2. EF-1αO cDNA    IVEMIPGKPM CVESFSDYPP LGRFAVRDMR QTVAVGVIKG VDKKAASSGK 450
3. EF-1αS cDNA    IVDMIPGKPM CVESFSDYPP LGRFAVRDMR QTVAVGVIKA VEKKAAGSGK
4. 42Sp50 cDNA    IITLKPIKPF CVERFFDYPP LGRFAARDLK QTVAVGVVKS VEHKAGAAAR

2. EF-1αO cDNA    VTKSAVKAGK -K  462
3. EF-1αS cDNA    VTKSAQKAAK TK
4. 42Sp50 cDNA    --RQVQKPVL VK
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*Figure 2.* Alignment of partial sequences of four peptides (*overlined*) derived from EF-1αO with sequences encoded by EF-1αO cDNA (Djé et al., 1990), EF-1αS cDNA (Krieg et al., 1989; Pötting et al., 1990), and 42Sp50 cDNA (Djé et al., 1990). The sequences of five partially overlapping peptides (*underlined*) derived from 42Sp50 (Viel et al., 1987) are also included in the comparison. The sequence of EF-1αO cDNA is identical to that of 42Sp48-1 cDNA (Coppard et al., 1991). Stars indicate positions at which the EF-1αO sequences (lines *1* and *2*) differ from the 42Sp50 sequences (lines *4* and *5*). Filled circles indicate positions at which the EF-1αO sequences (lines *1* and *2*) differ from the EF-1αS sequence (line *3*). Open circles indicate positions at which the EF-1αO cDNA sequence (line *2*) differs from the 42Sp50 protein sequence (line *5*). + indicates the only difference between the cDNA and protein sequences of 42Sp50 (lines *4* and *5*). X stands for unidentified residues.

42Sp48-1 cDNA (Coppard et al., 1991), but differ from the sequences encoded by EF-1αS cDNA and 42Sp50 cDNA in 3 and 8 positions, respectively (Fig. 2). The excellent agreement between the 42Sp50 cDNA and protein sequences (Fig. 2) justifies our previous identification of this cDNA clone (Djé et al., 1990). There can be no doubt that Coppard et al. (1991) misidentified the cDNAs they studied.

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