mRNA encoding the translation initiation factor eIF-4E is expressed early in *Xenopus* embryogenesis

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Abstract The translation initiation factor eIF-4E plays an important role in regulating the overall rate of translation in eukaryotic cells. To investigate the expression of eIF-4E itseIF, we characterized the eIF-4E mRNA expressed in *Xenopus* embryos. 5′-RACE was performed to determine the 5′-end of the mRNA and the result predicts isoforms differing at the amino-terminal end. Expression of the eIF-4E mRNA in *Xenopus* oocytes and embryos was examined by RT-PCR. *Xenopus* eIF-4E mRNA is produced during oogenesis and persists during the early stages of embryogenesis as a maternal mRNA.

Key words: Translation initiation factor; eIF-4E; mRNA; 5′-RACE; RT-PCR; *Xenopus laevis*

1. Introduction

Most of the eukaryotic mRNAs have the m^7G-containing cap structure at their 5′-termini [1]. Eukaryotic translation initiation factor eIF-4E is an mRNA cap-binding protein and is involved in the binding of mRNA to the 43S initiation complex to form the 48S initiation complex [2]. eIF-4E plays a crucial role in the initiation stage of protein synthesis. In the rabbit reticulocyte system, eIF-4E binds to eIF-4A and p220 to make a complex eIF-4F. The activity of eIF-4E is physiologically regulated by phosphorylation at Ser-53 [2]. cDNAs or genes for eIF-4E have been cloned from yeast [3], mouse [4], rabbit [5], human [6] and wheat [7,8]. The amino acid sequence as deduced from the cDNA clones predicts the presence of 8 tryptophans (9 in wheat eIF-4E) that are evolutionarily remarkably conserved in number and position. There is a report that the tryptophans would participate in cap recognition [9]. eIF-4E has been suggested to play a regulatory role in the control of cell growth in both mammalian [10,11] and yeast cells [12]. Using mouse eIF-4E mRNA, eIF-4E was reported to be involved in mesoderm induction in *Xenopus laevis* [13]. Recently, eIF-4E binding proteins 4E-BP1 and 4E-BP2 that can block cap-dependent translation were found in human cells [14,15]. They are suggested to link protein synthesis to the cell-signaling paths, also including insulin.

To be able to understand the protein synthesis in eukaryotes, it is important to investigate how the expression of eIF-4E itseIF is controlled. Here we report the cDNA sequence and characterization of mRNA encoding eIF-4E expressed in *Xenopus* embryos.

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2. Materials and methods

2.1. *Cloning and sequencing of* Xenopus *eIF-4E cDNA*

PCR was performed on oligo(dT)-primed λzapII *Xenopus laevis* tailbud cDNA library [16] using *Taq* DNA polymerase (Boehringer Mannheim) and primers derived from the rabbit eIF-4E cDNA sequence (corresponding to regions P1 and P2, see Fig. 1). Plaque hybridization was performed according to standard methods [17] using the PCR amplified cDNA fragment as a probe. More than 1×10^4 phages were screened and positive clones were isolated. Their inserted cDNAs were subcloned into the SK(−) form of the Bluescript vector. Both strands of the cDNA were sequenced using Pharmacia cycle sequencing kit or using TAKARA *Bca*BEST dideoxy sequencing kit on a Hitachi automated DNA sequencer WS-10A.

2.2. *Preparation of eggs and embryos of* Xenopus laevis

Mature male and female frogs were purchased from Johfu. An adult female was sacrificed and the ovary was obtained. The ovary was treated with 1 mg/ml collagenase for 15 h at 21°C, and then small oocytes (stage I and II) and large oocytes (stage V and VI) [18] were collected. *X. laevis* embryos were obtained by in vitro fertilization [19] and cultured in 0.1 × Steinberg solution after being dejellied in 2% cysteine. Staging was carried out according to Nieuwkoop and Faber [20].

2.3. *5′ and 3′-RACE*

RNAs were extracted from tailbud embryos by AGPC method [21] and treated with DNaseI (Boehringer Mannheim). 5′ and 3′-RACE was performed essentially as in [22] using the primers specific to *Xenopus laevis* eIF-4E sequence (Fig. 1). The amplified cDNA fragments were subcloned into pUC18. Several clones were sequenced as described above.

2.4. *RT-PCR*

RNAs were extracted from oocytes and embryos at various stages by the phenol method [23]. After treating with DNaseI, 1 μg of total RNA was reverse transcribed with Superscript II (Gibco-BRL) using random hexamers (Takara Shuzo) as primers. PCR amplification was performed for 30 cycles using 1/200 of the RT-mixture, the primers indicated in the figure legends and *Taq* DNA polymerase (Boehringer Mannheim). PCR conditions were as follows; denatured at 94°C for 1 min, annealing at 48°C for 1 min, and extension at 72°C for 3 min.

3. Results and discussion

PCR was performed on the *Xenopus laevis* tailbud cDNA library [16] using the primers derived from the rabbit eIF-4E cDNA sequence. The resultant PCR fragment of approximately 380 bp in length was cloned into pUC18 and sequenced. The sequence was highly homologous to the 3′-half part of the coding region of the rabbit eIF-4E cDNA. Screening *Xenopus laevis* tailbud cDNAs (more than 1×10^4 phages) with the PCR clone yielded two positive clones. The length of the inserted cDNAs were approximately 2.5 kbp and 0.6 kbp. The 2.5 kbp cDNA was subcloned into the SK(−) form of the Bluescript vector. Both strands of the cDNA were sequenced. The se-

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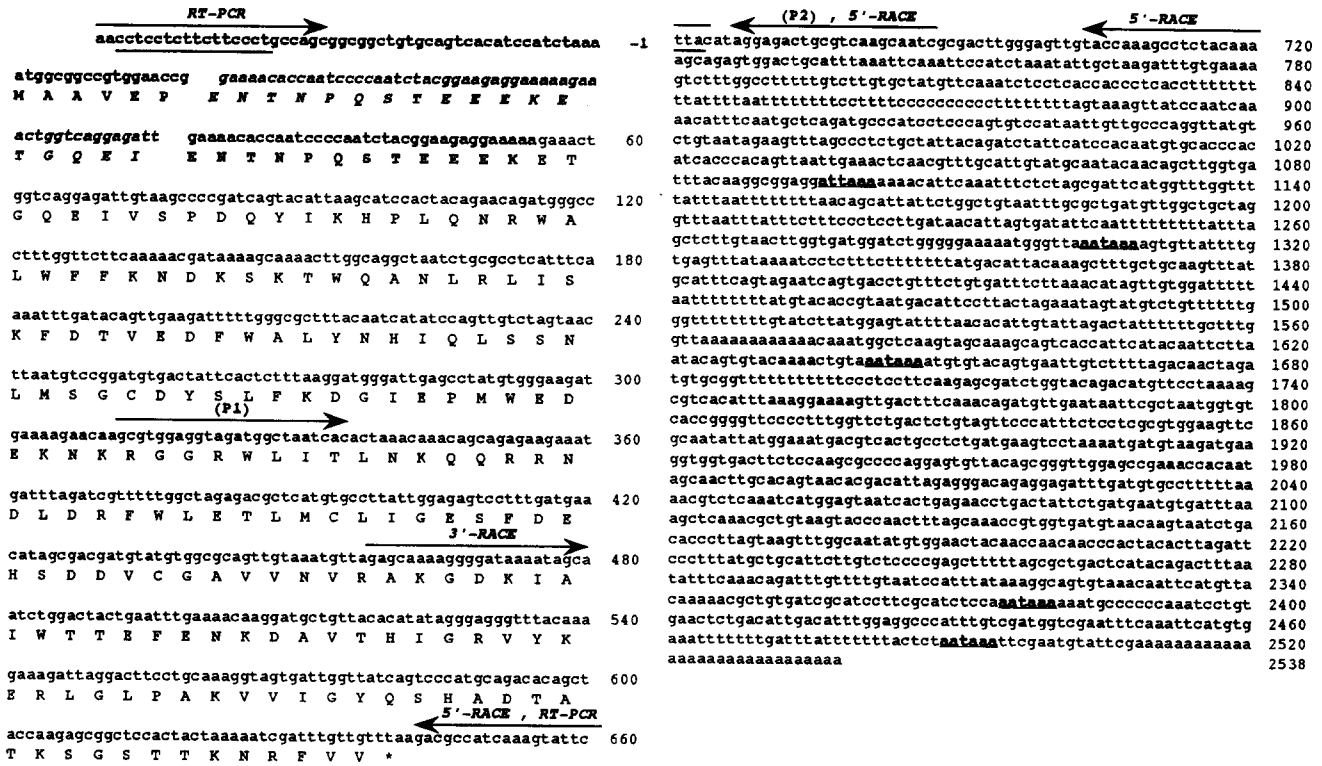

Fig. 1. cDNA and deduced amino acid sequences of *Xenopus laevis* eIF-4E. The arrows indicate the region corresponding to the primers used for PCR, RT-PCR, 5′ and 3′-RACE. The sequence obtained by 5′-RACE is printed in bold. The italicized sequence is included in the long 5′-RACE fragment. The oligopyrimidine tract in the 5′-region is underlined. Nucleotide positions are numbered according to the short type cDNA. Poly(A) signals are printed in bold and underlined.

quences of the cDNA and the deduced amino acids are presented in Fig. 1. The cDNA encodes a polypeptide which is homologous to the mammalian eIF-4E but missing the amino-terminal region.

5′-RACE (rapid amplification of cDNA ends) was used to obtain the sequences of the N-terminal coding region and the 5′-untranslated region. Consequently, 2 types of 5′-end fragments were obtained (Fig. 1). In one type, the 18-amino acids sequence ENTNPQSTEEEKETGQEI is repeated, but it is not repeated in the other. By RT-PCR using tailbud cDNA and the primers corresponding to the 24 bp sequence of the 5′-end region (Fig. 1, position −52 to −29) and the 22 bp sequence of the 3′-untranslated region (Fig. 1, position 643 to 664), we obtained two different clones having a length of 716 and 770 bp, respectively. Thus, the existence of the 2 types of cDNAs was confirmed (data not shown).

From the results described above, it is speculated that *Xenopus laevis* eIF-4E protein consists of 213 amino acids or 231 amino acids. Fig. 2 shows the alignment of eIF-4E protein. The overall homology of the amino acid sequence of *Xenopus* eIF-4E is more than 80% compared to the human, rabbit and mouse versions, albeit in the amino-terminal region, sequence homology is rather low. The large proportion of charged amino acids is characteristic for the amino-terminal region. The number and the positions of the tryptophan residues are highly conserved. In *Xenopus* eIF-4E, there are 3 cysteine residues, although mammalian eIF-4E contains 4 residues. Thus the amino acid residue at the fourth cysteine (TGT) in the latter is converted to phenylalanine (TTT) in the former. It is suggested that one pair of cysteine residues forms a disulfide bridge [2]. In mammalian eIF-4E, the fourth cysteine may exist as a sulfhydryl.

Both types of 5′-RACE fragments obtained as above contain

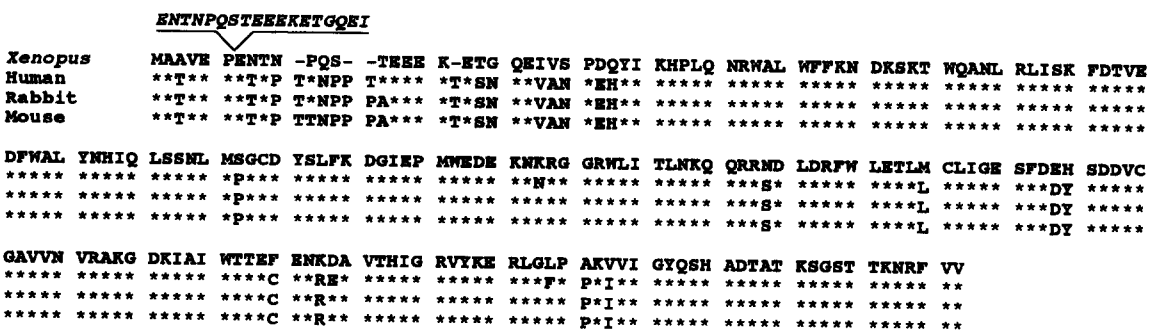

Fig. 2. Alignment of the deduced amino acid sequences for *Xenopus laevis*, human [6], rabbit [5] and mouse [4] eIF-4E protein. The italicized sequence is included in the long type cDNA. Identical and absent amino acid residues are indicated with asterisks and hyphens, respectively.

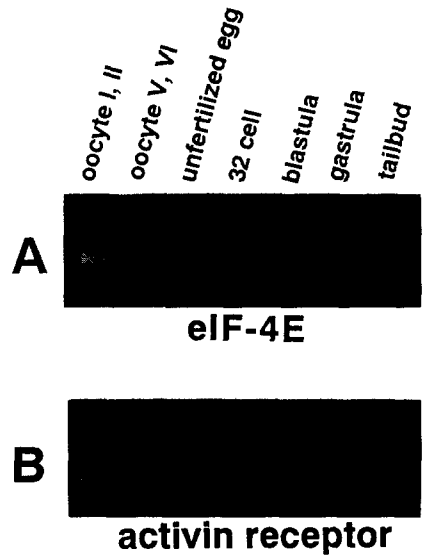

Fig. 3. Expression of *Xenopus* eIF-4E (A) and activin receptor (B) mRNAs in oocytes and embryos. Total RNAs extracted from oocytes and embryos at the indicated stages were analyzed by RT-PCR. PCR product was fractionated on 1.0% agarose gel electrophoresis. Amplified bands were visualized by ethidium bromide with ultraviolet light. Primers to eIF-4E mRNA are indicated in Fig. 1. Activin receptor primers are as follows: forward, GGAAAAAAACATGGGAGCTG (corresponding to the −10 to +10 region); reverse, CAAAACGAGAAGAGAACAAT (corresponding to the +460 to +480 region) [28].

approximately 50 bp 5′-untranslated sequence (Fig. 1). Thus, the 5′-untranslated region of *Xenopus* eIF-4E is 2.5 times longer than the human and the mouse versions. The 5′-terminal region contains characteristic oligopyrimidine tract CCTCCTCTTCTTCCCT which is similar to the oligopyrimidine tracts at the 5′ ends of the cDNAs for the vertebrate ribosomal proteins. In mammalian and *Xenopus*, the 5′-terminal oligopyrimidine elements have been shown to be involved in translational control especially at the step of polysomal mobilization [24–26]. In *Xenopus*, mRNA of the elongation factor EF-1α also has the oligopyrimidine element and the translation of the EF-1α appears to be coordinated with that of ribosomal proteins [27]. In the case of *Xenopus* eIF-4E cDNA, the first pyrimidine base cytosine is preceded by a purine base adenine. However, considering that reverse transcriptase can add a few extra template-independent nucleotides, the 5′-terminal base can be cytosine. The expression of *Xenopus* eIF-4E could be regulated at translation step coordinately with ribosomal protein and EF-1α. This is an attractive and interesting possibility.

The 3′-untranslated region (1866 nt) is nearly three times as long as the coding region (Fig. 1). This is a pyrimidine-rich region and contains many short repeats of thymine residues. It is of interest that there is a 31 nt pyrimidine sequence at position 850. The 3′-untranslated region contains 5 poly(A) signals; AATAAA at positions 1303, 1641, 2375, 2489, and ATTAAA at position 1096. In tailbud embryos, expression of the two shorter mRNAs generated by the use of different polyadenylation signals (polyadenylated at positions 1118 and 1328, Fig. 1) was confirmed by 3′-RACE (data nog shown).

Expression of the eIF-4E mRNA in oocytes and embryos was examined by RT-PCR. Fig. 3A shows that *Xenopus* eIF-4E mRNA is expressed at a high level in oocytes stage I–II, and the mRNA persists in early embryos. High levels of mRNA expression at the early phases of oogenesis is a well known phenomenon. Expression of activin receptor mRNA was also examined as a control (Fig. 3B). This mRNA is reported to be expressed as a maternal mRNA and its level is relatively constant throughout early *Xenopus* embryogenesis [28]. The expression pattern of the eIF-4E mRNA is similar to that of activin receptor mRNA during oogenesis, although it is somewhat different in later embryonic stages. It appears that the amount of the eIF-4E mRNA gradually decreases from cleavage stage to gastrula stage and turn to increase at tailbud stage. Further experiments are now under way.

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References

[1] Furuichi, Y. and Miura, K. (1975) Nature 253, 374–375.
[2] Rhoads, R.E., Joshi-Brave, S. and Rinker-Schaeffer, C. (1993) in: Progress in Nucleic Acid Research and Molecular Biology, Academic Press, pp. 183–219.
[3] Altmann, M., Handschin, C. and Trachsel, H. (1987) Mol. Cell. Biol. 3, 998–1003.
[4] Jaramillo, M., Pelletier, J., Edery, I., Nielsen, P.J. and Sonenberg, N. (1991) J. Biol. Chem. 266, 10446–10451.
[5] Rychlik, W. and Rhoads, R.E. (1992) Nucleic Acids Res. 20, 6415.
[6] Rychlik, W., Domier, L.L., Gardner, P.R., Hellmann, G. and Rhoads, R.E. (1987) Proc. Natl. Acad. Sci. USA 84, 945–949.
[7] Metz, A.M. Timmer, R.T. and Browning, K.S. (1992) Nucleic Acids Res. 20, 4096.
[8] Allen, M.L., Metz, A.M., Timmer, R.T., Rhoads, R.E. and Browning, K.S. (1992) J. Biol. Chem. 267, 23232–23236.
[9] Ishida, T., Katsuta, M., Inoue, M., Yamagata, Y. and Tomita, K. (1983) Biochem. Biophys. Res. Commun. 115, 849–854.
[10] Lazaris-Karatzas, A., Montine, K.S. and Sonenberg, N. (1990) Nature 345, 544–547.
[11] De Benedetti, A. and Rhoads, R.E. (1990) Proc. Natl. Acad. Sci. USA 87, 8212–8216.
[12] Brenner, C., Nakayama, N., Goebl, M., Tanaka, K., Toh-e, A. and Matsumoto, K. (1988) Mol. Cell. Biol. 8, 3556–3559.
[13] Klein, P.S. and Melton, D.A. (19940 Science 265, 803–806.
[14] Pause, A., Belsham, G.J., Gingras, A., Donze, O., Lin, T., Lawrence Jr, J.C. and Sonenberg, N. (1994) Nature 371, 762–767.
[15] Lin, T., Kong, X., Haystead, A.J., Pause, A., Belsham, G., Sonenberg, N. and Lawrence Jr., J.C. (1994) Science 266, 653–656.
[16] Tashiro, K., Yamada, R., Asano, M., Hashimoto, M., Muramatsu, M. and Shiokawa, K. (1991) Biochem. Biophys. Res. Commun. 174, 1022–1027.
[17] Sambrook, J., Fritsch, E.F. and Maniatis, T. (1989) Molecular Cloning: A Laboratory Mannual, second edition, Cold Spring Harbor, NY.
[18] Dumont, J.N. (1972) J. Morph. 136, 153–180.
[19] Heasman, J., Holwill, S. and Wylie, C.C. (1991) Methods Cell Biol. 36, 213–230.
[20] Nieuwkoop, P.D. and Faber, J. (1969) Normal Table of *Xenopus laevis* (Daudin), North Holland Publishing Co., Amsterdam.
[21] Chomczynski, P. and Sacchi, N. (1987) Anal. Biochem. 162, 156–159.
[22] Frohman, M.A. (1993) Methods Enzymol. 218, 340–356.
[23] Ruizi, Altaba, A. and Melton, D.A. (1989) Development 106, 173–183.
[24] Levy, S., Avni, D., Hariharan, N., Perry, R.P. and Meyuhas, O. (1991) Proc. Natl. Acad. Sci. USA 88, 3319–3323.
[25] Mariottini, P. and Amaldi, F. (1990) Mol. Cell. Biol. 10, 816–822.
[26] Kaspar, R.L., Tomohito, K., Cranston, H., Morris, D.R. and White, M.W. (1992) J. Biol. Chem. 267, 508–514.
[27] Loreni, F., Francesconi, A. and Amaldi, F. (1993) Nucleic Acids Res. 21, 4721–4725.
[28] Kondo, M., Tashiro, K., Fujii, G., Asano, M., Miyoshi, R., Yamada, R., Muramatsu, M. and Shiokawa, K. (1991) Biochem. Biophys. Res. Commun. 181, 684–690.