Reviews

Removal of part of the eEF1A GTP binding domain induced translation errors *in vitro*

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Eukaryotic translation elongation factor 1A (eEF1A) is one of the main components of the translation machinery acting at the elongation step. Oncogene PTI-1 which is expressed during prostate cancer development, codes the protein, homologous to eEF1A. In this paper the hypothesis is tested that the product of PTI-1 gene can influence the accuracy of translation. The ability of the PTI-1 protein analogue to stimulate misincorporation of leucine by ribosomes programmed with poly(U) was demonstrated. This finding favors the hypothesis about the ability of the PTI-1 protein to influence translation accuracy in vivo.

Keywords: *eukaryotic translation elongation factor 1A, oncogene PTI-1, ribosome, tRNA.*

Introduction. Eukaryotic translation elongation factor 1A (eEF1A) is a guanine nucleotide-binding protein which provides correct recognition of mRNA codon by tRNA anticodon on the ribosome. Besides its main role in translation, eEF1A may be also involved into other cell processes, such as signal transduction [1], cytoskeleton organization [2, 3], as well as apoptosis [4], carcinogenesis [5, 6], virus infection [7], diabetes [8, 9].

Recently more data on participation of eEF1A in carcinogenesis appear. In particular, the connection between eEF1A and oncogenesis was found at human prostate carcinoma research [10]. Dominant oncogene was found in tumor tissues, which was later called PTI-1(prostate tumor inducing gene-1). It was shown

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eEF1A1   1    MGKEKTHINI VVIGHVDSGK STTTGHLIYK CGGIDKRTIE KFEKEAAEMG
 eEF1A1       ..........................................................
PTI-1         ..........................................................
eEF1A1   51   KGSFKYAWVL DKLKAERERG ITIDISLWKF ETTKYYITII DAPGHRDFIK
 eEF1A1  1    ..........................G ITIDISLWKF ETTKYYITII DAPGHRDFIK
PTI-1    1    ..........................MQSERG ITIDISLWKF ETTKYYITII DAPGHRDFIK
eEF1A1   101  NMITGTSQAD CAVLIVAAGV GEFEAGISKN GQTREHALLA YTLGVKQLIV
 eEF1A1  32   NMITGTSQAD CAVLIVAAGV GEFEAGISKN GQTREHALLA YTLGVKQLIV
PTI-1    37   NMITGTSQAD CAVLIVAAGV GEFEAGISKN GQTREHALLA YTLGVKQLIV
eEF1A1   151  GVNKMDSTEP AYSEKRYDEI VKEVSAYIKK IGYNPATVPF VPISGWHGDN
 eEF1A1  82   GVNKMDSTEP AYSEKRYDEI VKEVSAYIKK IGYNPATVPF VPISGWHGDN
PTI-1    87   GVNKMDSTEP AYSEKRYDEI VKEVSAYIKK IGYNPATVPF VPISGWHGDN
eEF1A1   201  MLEPSPNMPW FKGWKVERKE GNASGVSLLE ALDTILPPTR PTDKPLRLPL
 eEF1A1  132  MLEPSPNMPW FKGWKVERKE GNASGVSLLE ALDTILPPTR PTDKPLRLPL
PTI-1    137  MLEPSPNMPW FKGWKVERKE GNASGVSLLE ALDTILPPTR PTDKPLRLPL
eEF1A1   251  QDVYKIGGIG TVPVGRVETG ILRPGMVVTF APVNITTEVK SVEMHHEALS
 eEF1A1  182  QDVYKIGGIG TVPVGRVETG ILRPGMVVTF APVNITTEVK SVEMHHEALS
PTI-1    187  QDVYKIGGIG TVPVGRVETG ILRPGMVVTF APVNITTEVK SVEMHHEALS
eEF1A1   301  EALPGDNVGF NVKNVSVKDI RRGNVCGDSK SDPPQEAAQF TSQVIILNHP
 eEF1A1  232  EALPGDNVGF NVKNVSVKDI RRGNVCGDSK SDPPQEAAQF TSQVIILNHP
PTI-1    237  EALPGDNVGF NVKNVSVKDI RRGNVCGDSK SDPPQEAAQF TSQVIILNHP
eEF1A1   351  GQISAGYSPV IDCHTAHIAC KFAELKEKID RRSGKKLEDN PKSLKSGDAA
 eEF1A1  282  GQISAGYSPV IDCHTAHIAC KFAELKEKID RRSGKKLEDN PKSLKSGDAA
PTI-1    287  GQISAGYSPV IDCHTAHIAC KFAELKEKID RRSGKKLEDN PKSLKSGDAA
eEF1A1   401  IVEMVPGKPM CVESFSQYPP LGRFAVRDMR GTVAVGVIKN VEKKSGGAGK
 eEF1A1  332  IVEMVPGKPM CVESFSQYPP LGRFAVRDMR GTVAVGVIKN VEKKSGGAGK
PTI-1    337  IVEMVPGKPM CVESFSQYPP LGRFAVRDMR GTVAVGVIKN VEKKSGGAGK
eEF1A1   451  VTKSAQKAQK AGK
 eEF1A1  382  VTKSAQKAQK AGK
PTI-1    387  VTKSAQKAQK AGK
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Fig.1 The comparison of amino acid sequences for native eEF1A, trypsin-modified eEF1A and protein PTI-1, derived from nucleotide sequence of the PTI-1 mRNA.

that PTI-1 gene contains the sequence of 630 nucleotide bases (n.b.) in 5 -not coding area, which is 87% identical to the sequence of 23S ribosome RNA of *Mycoplasma hyopneumoniae*. It is interesting that the coding sequence of this gene is 99.25% identical to the sequence of the eEF1A protein, shortened at N-end of 67 amino acid residues and with three additional amino acids added (Fig.1) [11]. Therefore, PTI-1 oncogene can code a protein with 398 a.a., which is the homologue of eEF1A. As the sequence absent in PTI-1 comparing to eEF1A is a part of GTP-binding site of translation elongation factor, it is logical to suppose that this modification may lead to changes in PTI-1 functioning compared with eEF1A.

Minding the mentioned above, the hypothesis has been put forward that PTI-1 represents the new oncogene class, the transformational properties of which may be mediated by the following mechanisms: 1) action on the translation accuracy, resulting in synthesis of “wrong” polypeptides, , in particular, the regulatory ones, which could effect tumor development [12]; 2) influence on cytoskeletal proteins due to the changes in a known ability of eEF1A to interact with actin and tubulin; 3) the changes in one or several

different pathways of signal transduction possibly involving PTI-1 as a G-protein [13].

It is known that eEF1A mutations may have direct influence on the reading frame shift and the increased incorporation of wrong amino acids in *Sacharomyces cerevisae*. For example, the substitution of only one amino acid in eEF1A sequence changes the level of selectivity and correct codon-anticodon interaction of aminoacyl-tRNA in the process of translation [14]. Therefore, the appearance in tumor cells of eEF1A homologue, which is coded by PTI-1, seems to be the reason for decrease of translation accuracy. The goal of this work was to test principal possibility of PTI-1 analogous protein to affect the translation accuracy and therefore, to investigate whether the protein is capable of increasing the misincorporation of leucine by ribosomes programmed with poly(U).

Materials and Methods. eEF1A and eEF2 preparations were obtained from rabbit liver as earlier described [15]. Preparations of rabbit liver ribosomal subunits and of bovine liver total tRNA were obtained as in [16].

Limited tryptic cleavage of rabbit liver eEF1A was performed as earlier described [15].

Aminoacylation of total tRNA was performed in the buffer - 30mM imidazole, pH 7.5 (Sigma, USA); 5mM $MgCl_2$, 100mM KCl, 3mM dithiothreitol (Amersham, Sweden); 3mM ATP, pH 7.0; 40 мМ [^{14}C]Phe (Amersham), 120мg of bovine liver total tRNA and 200мg of total aminoacyl-tRNA synthetase preparation purified from rabbit liver. Aminoacylation was performed for 15min at 371C, followed by cooling on ice bath. Aliquots of this mixture served as a source of aminoacyl-tRNA in the system of poly(U) translation.

Incorporation of [^{14}C]phenylalanine into product of poly(U) translation was performed at 371C in the buffer: 50mM HEPES, pH7.6 (Fluka, the Czech Republic), 8mM $MgCl_2$, 70mM KCl, 10% glycerol, 1mM ATP, pH7.0 (Sigma), 0.4mM GTP, pH7.5, 40мg/ml creatine phosphokinase (Serva), 10mM creatine phosphate (Serva), 10мg/sample of poly(U) (Sigma). Translational mixture (100мl) also contained 15 pmol 40S and 25 pmol 60S ribosomal subunits, 60 pmol eEF2, different quantities eEF1A or eEF1A, and 50 мl of mixture for aminoacylation containing 120 мg of total tRNA aminoacylated with [^{14}C]Phe. The reaction was stopped by adding 2ml of cooled TCA. Then the reaction mixture was heated (10min, 901C) for aminoacyl-tRNA destruction and cooled on ice bath. Precipitates were placed on GF/C filters (Whatman, USA) and rinsed by 30ml of cold 3% TCA.

Misincorporation of leucine to the poly(U) translation product was investigated in the same buffer as in the case with phenylalanine except for increasing $MgCl_2$ concentration up to 13mM.

The radioactivity of dry filters was measured in Optiphase scintillator using RackBeta meter 1219 (LKB, Sweden). Effectiveness of [^{14}C] calculation on filters was 71%, [^{3}H] – 26%.

Results and Discussion. Since the product of limited tryptic cleavage of eEF1A and hypothetical oncogene product PTI-1 are 99% identical (Fig.1), eEF1A, modified by tripsin, was used as a model of PTI-1 protein.

We have determined that eEF1A, regardless of the absence of 1/3 GTP/GDP-binding domain, preserved its functional activity in GTP, GDP, and tRNA binding to some degree, as well as it could maintain poly(U) translation in cell-free translation system, obtained from individual components [15]. The task of current work was to determine whether the presence of PTI-1 analogue protein in cell-free translation system influences the ability of the system to misincorporate leucine instead of phenylalanine during poly(U) translation.

Unlike in our early experiments [15], where the preparation of individual phenylalanine yeast tRNA was used for the measurement of the eEF1A translational activity, in this work we used the preparation of total tRNA of bovine liver as this preparation contained $tRNA^{Phe}$ and $tRNA^{Leu}$ which is the obligatory condition for the translational errors study in the poly(U) translational system.

The optimal concentration of eEF1A or eEF1A for poly(U) translation was determined (Fig. 2). It is important to state that, while using both yeast and bovine liver tRNA, even the addition of eEF1A extra amounts did not allow increasing the activity of cell-free translational system to the level which was observed in the presence of the native eEF1A, *i.e.* the splitting of almost 1/3 of GTP-binding eEF1A domain led to partial loss of the eEF1A activity as judged by estimation of the translation kinetics.

The kinetics of poly(U) translation with the participation of eEF1A and eEF1A was studied (Fig.3) using total tRNA aminoacylated with [^{14}C]Phe. The rate of incorporation of [^{14}C]phenylalanine into TCA insoluble precipitate in the presence of eEF1A was ~1.12pmoles Phe/min, and in the presence of eEF1A ~0.61pmoles Phe/min at 8mM Mg ions concentration in the reaction mixture.

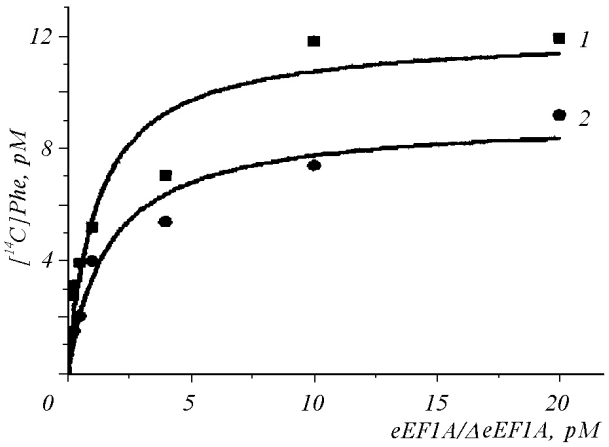

Fig.2 Polyphenylalanine synthesis at 8mM $MgCl_2$ in cell-free translation system at different eEF1A (1) and eEF1A (2)

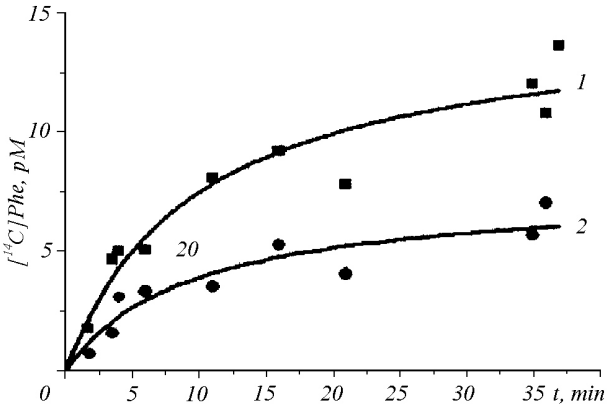

Fig. 3 The kinetics of polyphenylalanine synthesis in cell-free translation system in the presence of eEF1A (1) or eEF1A (2) (14 pM/sample) at 8mM $MgCl_2$ concentration.

It is known that leucine misincorporation into poly(U) translation product in cell-free protein-synthesizing systems form *Escherichia coli* [17] and wheat germs is induced by the increase in Mg^{2+} ions concentration [18]. However, since the optimal $MgCl_2$ concentration for translation error study in the protein-synthesizing system of higher eukaryotes remained not determined, we investigated the influence of Mg^{2+} concentration on the phenylalanine and leucine incorporation into the product of poly(U) translation (Fig.4). The highest level of leucine misincorporation during poly(U) translation in this system was observed at $MgCl_2$ concentrations from 12 to 14mM.

The translation error index was determined as the ratio of misincorporated leucine to incorporated phenylalanine. The incorporation of amino acids into

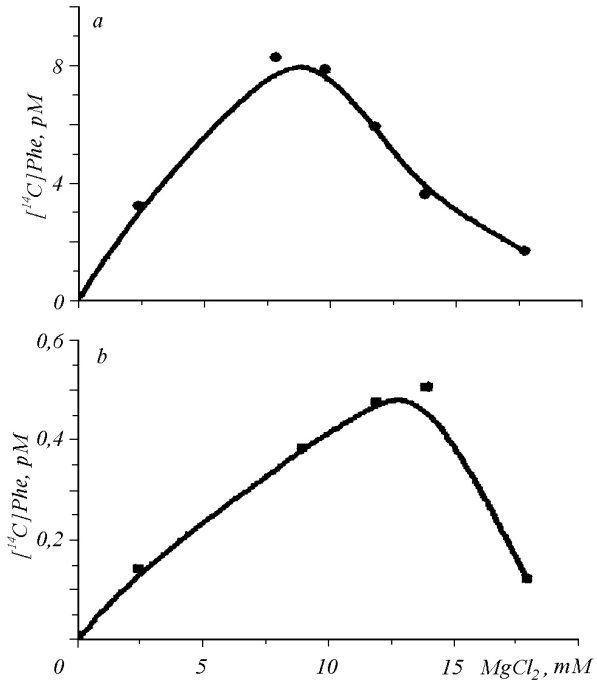

Fig. 4 The dependence of incorporation of [^{14}C]Phe (a) and [^{3}H]Leu (b) in TCA insoluble precipitate from $MgCl_2$ in the presence of full-sized eEF1A.

TCA-insoluble product was studied at initial rate of poly(U) translation by 80S ribosomes. 4min incubation time fulfilled completely the initial rate conditions and allowed obtaining the sufficient amount of radioactivity in TCA-precipitate. The experimental results are shown in the Table. As it is seen, the replacement of eEF1A for eEF1A in the translation system leads to almost 6 times increase in the level of translation errors.

Therefore, the obtained data are in favor of the hypothesis considering the possibility of translational errors induction at the presence of the PTI-1 protein product. It is not excluded, that such mechanism may explain known translation errors increase during oncogenesis [10].

As it was mentioned above, first 67 amino acid residues are absent in PTI-1 protein in the comparison to eEF1A. How can it influence the translation errors? On the example of yeast eEF1A mutagenesis the involvement of amino acid residues Asn153 and Asp156 [20], as well as Glu122 and Thrl42 [21], to the control of accuracy has been shown. It is interesting that according to the X-ray analysis data, all these residues are located near or participate directly in the formation of GDP-binding “pocket” of eEF1A. These residues provide the interaction with GDP guanine ring, at the same time, on the basis of X-ray analysis, amino

Factor	Initial rate of incorporation of [^{14}C]Phe, pM/min	Initial rate of misincorporation of [^{3}H]Leu, pM/min	Translation error index ([^{3}H]Leu/[^{14}C]Phe)
eEF1A	1.12	0.11	0.1
eEF1A	0.61	0.35	0.57

acids may interact with the phosphate group of the same GDP molecule in the area between the 15th and 19th eEF1A residues [22], *i.e.* those amino acids that are not present in eEF1A or in PTI-1 protein. It is logical to assume that if this part of GDP-binding site is absent in eEF1A, then the affinity of the mentioned protein for GDP be decreased. That notion is supported by our data and the data of scientific literature [15, 23]. Therefore, the decreased GDP/GTP binding capability of the PTI-1 protein may contribute to the increase of error level.

Therefore, we show for the first time that the loss of the part of GTP-binding domain, responsible for binding of the GTP/GDP phosphate groups, may be important for correct eEF1A functioning in translation. The results of work show that eEF1A, used as a model of PTI-1 protein product, is capable to participate in the protein biosynthesis *in vitro*, however causing the increase in misincorporation of amino acids by ribosomes. Therefore, it could not be excluded that the appearance of the PTI-1 protein in tumor cells may lead to the synthesis of erroneous proteins with altered functions or no function at all. This is in favor of translational mechanism of the PTI-1 oncogene participation in carcinogenesis. Elucidation of the possible participation of PTI-1 in the induction of translational errors *in vivo* is the task for further investigations.

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Удаление части GTP-связывающего домена эукариотного фактора элонгации трансляции 1А приводит к индукции трансляционных ошибок in vitro

Резюме

Эукариотный фактор элонгации трансляции 1А(eEF1A) является одним из главных компонентов аппарата белкового синтеза на стадии элонгации. Онкоген PTI-1, экспрессия которого обнаружена при раке предстательной железы человека, кодирует белок - гомолог eEF1A. Проверена гипотеза о том, что белковый продукт гена PTI-1 влияет на точность трансляции. Продемонстрирована способность аналога PTI-1 стимулировать ошибочное включение лейцина рибосомами, программированными поли(U)-матрицей, что подтверждает возможность продукта гена PTI-1 влиять на точность трансляции in vivo.

Ключевые слова: эукариотный фактор элонгации трансляции 1А, онкоген PTI-1, рибосома мРНК.

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