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Short communication

# A2 isoform of mammalian translation factor eEF1A displays increased tyrosine phosphorylation and ability to interact with different signalling molecules

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**Abstract**

The eEF1A1 and eEF1A2 isoforms of translation elongation factor 1A have 98% similarity and perform the same protein synthesis function catalyzing codon-dependent binding of aminoacyl-tRNA to 80S ribosome. However, the isoforms apparently play different non-canonical roles in apoptosis and cancer development which are awaiting further investigations. We hypothesize that the difference in non-translational functions could be caused, in particular, by differential ability of the isoforms to be involved in phosphotyrosine-mediated signalling.

The ability of eEF1A1 and eEF1A2 to interact with SH2 and SH3 domains of different signalling molecules *in vitro* was compared. Indeed, contrary to eEF1A1, eEF1A2 was able to interact with SH2 domains of Grb2, RasGAP, Shc and C-terminal part of Shp2 as well as with SH3 domains of Crk, Fgr, Fyn and phospholipase C-gamma1.

Interestingly, the interaction of both isoforms with Shp2 *in vivo* was found using stable cell lines expressing eEF1A1-His or eEF1A2-His. The formation of a complex between endogenous eEF1A and Shp2 was also shown. Importantly, a higher level of tyrosine phosphorylation of eEF1A2 as compared to eEF1A1 was demonstrated in several independent experiments and its importance for interaction of eEF1A2 with Shp2 *in vitro* was revealed.

Thus, despite the fact that both isoforms of eEF1A could be involved in the phosphotyrosine-mediated processes, eEF1A2 apparently has greater potential to participate in such signalling pathways. Since tyrosine kinases/phosphatases play a prominent role in human cancerogenesis, our observations may gave a basis for recently found oncogenicity of the eEF1A2 isoform.


*Keywords:* eEF1A1; eEF1A2; SH2 domain; SH3 domain; Shp2

## 1. Introduction

Translation elongation factor 1A (eEF1A) is one of the core members of the elongation machinery providing high efficiency and processivity of the protein synthesis process in the eukaryotic cell (Negrutskii & El'skaya, 1998). eEF1A forms a ternary complex with aminoacyl-tRNA and GTP and delivers the correct aminoacyl-tRNA to the A site of mRNA programmed ribosome in the GTP hydrolysis-dependent mode. The GDP-bound form of eEF1A has been shown to interact with deacylated tRNA (Petrushenko et al., 1997) and can transport it

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to aminoacyl-tRNA synthetase as the tRNA recycling arm of a tRNA channelling cycle (Petrushenko, Shalak, Budkevich, Negruskii, & El'skaya, 2002).

It is believed that the translation function of eEF1A might be combined with its involvement in cytoskeletal network maintenance and various signalling pathways (Lamberti et al., 2004). eEF1A has been shown to interact with phospholipase C-gamma1 (PLC-gamma1) (Chang et al., 2002; Kim et al., 1999) and non-receptor tyrosine kinase Txk of the Tec family (Maruyama, Nara, Yoshikawa, & Suzuki, 2007). Importantly, Txk can form a complex with poly(ADP-ribose) polymerase 1 and eEF1A to influence interferon-gamma gene transcription in Th1 cells. Recently Lau, Castelli, Lin, and Macaulay (2006) identified eEF1A as a novel binding partner for Akt2 rho-associated kinase. In most cases it is not known whether interactions of eEF1A with protein kinase might lead to phosphorylation of eEF1A. Neither has the role of eEF1A in signal transduction pathways been identified.

In mammalian cells, two tissue- and development-specific isoforms, eEF1A1 and eEF1A2, are present. The expression of the 98% similar isoforms is mutually exclusive (Kahns et al., 1998). Interestingly, eEF1A2, which is present normally only in muscles and neurons, was recently associated with tumor development in tissues that normally express only eEF1A1 (Amiri et al., 2007; Anand et al., 2002). How eEF1A2 isoform is involved in malignant transformation is not as yet understood. Since tyrosine kinases are prominent players in cancer development, direct comparison of the ability of eEF1A1 and eEF1A2 to be involved in phosphoTyr-specific signalling processes could help to interpret cancer-related properties of eEF1A2.

In this study, we compared for the first time the interactions of eEF1A1 and eEF1A2 with SH2 and SH3 domains of various signalling molecules. Contrary to eEF1A1, eEF1A2 was able to interact with SH2 domains of Grb2, RasGAP, Shc and Shp2 as well as with SH3 domains of Crk, Fgr, Fyn and PLC-gamma1. Both eEF1A1 and eEF1A2 formed complexes with SH2 domain of PLC-gamma1. *In vivo* interaction between endogenous eEF1A and tyrosine phosphatase Shp2 was confirmed in HEK293 cell line and tyrosine phosphorylation of eEF1A1 and eEF1A2 was demonstrated by Western blots with anti-phosphotyrosine-specific antibodies. Importantly, the level of phosphorylation of eEF1A2 was higher than eEF1A1 when isolated from tissues, and also when overexpressed in cells. Furthermore, the level of phosphorylation has been shown to be crucial for *in vitro* complex formation of eEF1A2 and SH2 domain of Shp2.

## 2. Materials and methods

### 2.1. Cell lines and plasmid constructs

HEK293 cells were purchased from the American Type Culture Collection (Manassas, VA) and grown according to their instructions. To produce eEF1A1 and eEF1A2 stable cell lines pcDNA 3.1 eEF1A1 and eEF1A2 with the C-terminal His-tags were linearized by MunI and transfected in HEK293 cells using ExGene500 (Fermentas) according to manufacturer recommendations. G418 was used to select stable cell lines.

### 2.2. Bioinformatics

ScanSite Program was used for bioinformatic identification of possible SH2 and SH3 domains binding sites within eEF1A1 and eEF1A2. ScanSite employs the matrix of selectivity values for amino acids at each position relative to an orienting residue as determined by the oriented peptide library technique (Obenauer, Cantley, & Yaffe, 2003).

### 2.3. Purification of the eEF1A1 and eEF1A2 isoforms

eEF1A1 was purified from rabbit liver using a combination of the gel filtration, anion exchange, cation exchange and hydroxyapatite chromatographies as described previously (Budkevich et al., 2002). eEF1A2 was isolated from rabbit muscles using the same procedure except the gel-filtration step was omitted in some cases. To confirm the biological activity of the isolated isoforms, the GDP/[$^3$H]GDP exchange was carried out as in (Carvalho, Carvalho, & Merrick, 1984).

### 2.4. Pull down assay

Purified GST-SH2 and GST-SH3 domains of various signalling molecules were kindly provided by Prof. Ivan Gout (London, UK) (Gout et al., 1993). In a pull down assay, 1.5 μg of GST, GST-SH2 or GST-SH3 domains fusion protein was incubated with glutathione-Sepharose 4B beads (Amersham Biosciences) for 2 h at 4 °C. The beads were then washed to remove unbound proteins and incubated at 4 °C for 3 h with 1.5 μg of purified eEF1A1 or eEF1A2. Non-specific interactions were removed by extensive washing and bound proteins eluted by boiling in Laemmli sample buffer. Eluted proteins were resolved by SDS-PAGE, transferred to polyvinylidene difluoride membrane, and detected by immunoblotting with specific antibodies.