The International Journal of Biochemistry & Cell Biology 69 (2015) 105–113

Contents lists available at ScienceDirect

The International Journal of Biochemistry & Cell Biology

journal homepage: www.elsevier.com/locate/biocel

# Elongation factor-1A1 is a novel substrate of the protein phosphatase 1-TIMAP complex

Anita Boratkó, Margit Péter, Zsófia Thalwieser, Előd Kovács, Csilla Csortos*

*Department of Medical Chemistry, Faculty of Medicine, University of Debrecen, Egyetem tér 1, Debrecen H-4032, Hungary*

ARTICLE INFO

*Article history:*
Received 28 July 2015
Received in revised form 18 October 2015
Accepted 19 October 2015
Available online 21 October 2015

*Keywords:*
Endothelial cell
eEF1A1
Protein phosphatase 1
TIMAP

ABSTRACT

TIMAP (TGF-β inhibited membrane associated protein) is a protein phosphatase 1 (PP1) regulatory subunit highly abundant in endothelial cells and it is involved in the maintenance of pulmonary endothelial barrier function. It localizes mainly in the plasma membrane, but it is also present in the nuclei and cytoplasm. Direct interaction of TIMAP with the eukaryotic elongation factor 1 A1 (eEF1A1) is shown by pull-down, LC–MS/MS, Far-Western and immunoprecipitations. In connection with the so called moonlighting functions of the elongation factor, eEF1A is thought to establish protein–protein interactions through a transcription-dependent nuclear export motif, TD-NEM, and to aid nuclear export of TD-NEM containing proteins. We found that a TD-NEM-like motif of TIMAP has a critical role in its specific binding to eEF1A1. However, eEF1A1 is not or not exclusively responsible for the nuclear export of TIMAP. On the contrary, TIMAP seems to regulate membrane localization of eEF1A1 as the elongation factor co-localized with TIMAP in the plasma membrane fraction of control endothelial cells, but it has disappeared from the membrane in TIMAP depleted cells. It is demonstrated that membrane localization of eEF1A1 depends on the phosphorylation state of its Thr residue(s); and ROCK phosphorylated eEF1A1 is a novel substrate for TIMAP-PP1 underlining the complex regulatory role of TIMAP in the endothelium. The elongation factor seems to be involved in the regulation of endothelial cell attachment and spreading as silencing of eEF1A1 positively affected these processes which were monitored by transendothelial resistance measurements.
© 2015 Elsevier Ltd. All rights reserved.

## 1. Introduction

Endothelium has a central role in inflammation, hemostasis, vasoregulation, angiogenesis, and vascular growth. TIMAP, *T*GF-β *i*nhibited *m*embrane *a*ssociated *p*rotein, (PPP1R16B) is highly abundant in endothelial cells (EC) (Cao et al., 2002). The *N*-terminal half of TIMAP contains a protein phosphatase 1 (PP1) binding motif (KVSF) followed by several ankyrin repeats. Based on this structural feature, TIMAP was referred as a member of the MYPT family of PP1 regulatory subunits (Ito et al., 2004). Earlier, we showed that endogenous TIMAP prefers binding to the β isoform (aka δ isoform) of the catalytic subunit of PP1 (PP1c) in human pulmonary EC, and the interaction between PP1c and TIMAP was characterized (Csortos et al., 2008; Shopik et al., 2013). Moreover, the involvement of TIMAP in maintaining pulmonary EC barrier function was demonstrated, as the absence of TIMAP enhanced the barrier compromising effects of thrombin and nocodazole, and attenuated the effects of the barrier protecting sphingosine-1-phosphate and ATP. In agreement with the membrane-associated localization of TIMAP, our results indicated that TIMAP mediates the phosphorylation level of ERM (ezrin-radixin-moesin) proteins via regulation of PP1c in EC (Czikora et al., 2011; Csortos et al., 2008). Further, direct interaction of TIMAP with the non-integrin laminin receptor-1 (LAMR1) and the involvement of TIMAP in the regulation of LAMR1 phosphorylation level have been reported (Kim et al., 2005; Shopik et al., 2013).

Prenylation of TIMAP is required for its localization at the cell membrane (Cao et al., 2002). We found interaction between TIMAP and RACK1 (receptor for activated C-kinase 1), an adaptor for multiple proteins. They co-localize in the cytoplasm close to the nucleus in EC. Identification of farnesyl transferase, as another novel interacting partner of RACK1 indicated that transient parallel linkage

*Abbreviations:* BPAEC, bovine pulmonary artery endothelial cell; EC, endothelial cell; ECIS, electric cell-substrate impedance sensing; eEF1A1, elongation factor 1A1; ERM, ezrin-radixin-moesin; GST, glutathione S-transferase; HPAEC, human pulmonary artery endothelial cells; LAMR1, laminin receptor-1; LC–MS/MS, liquid chromatography-tandem mass spectrometry; MYPT, myosin phosphatase targeting subunit; RACK1, receptor for activated C kinase 1; ROCK, Rho-associated protein kinase; PP1, protein phosphatase 1; PP1c, catalytic subunit of protein phosphatase 1; TIMAP, TGF-β inhibited membrane associated protein; TD-NEM, transcription-dependent nuclear export motif.

* Corresponding author. Tel.: +36 52412345x61173; fax: +36 52412566.
*E-mail address:* csortos@med.unideb.hu (C. Csortos).

http://dx.doi.org/10.1016/j.biocel.2015.10.021
1357-2725/© 2015 Elsevier Ltd. All rights reserved.

of TIMAP and farnesyl transferase to RACK1 may ensure prenylation and subsequent transport of TIMAP to the plasma membrane where it may attend in maintaining the endothelial barrier as a phosphatase regulator (Boratko et al., 2013).

On the other hand, a bipartite nuclear localization signal (NLS) is also present in TIMAP and accordingly, it appears in the nucleus of EC as well (Csortos et al., 2008). However, the significance of nuclear localization and trafficking of TIMAP between the cytoplasm and the nucleus is still obscure.

Our new findings indicate an interaction between TIMAP and the eukaryotic elongation factor 1 A1 (eEF1A1). The mainly cytosolic eEF1A1 is expressed in all mammalian tissues except for skeletal muscle and heart where instead the highly homologous eEF1A2 is expressed (Kahns et al., 1998). The 462 amino acid residues long protein contains many potential phosphorylation sites, but only few of those have been experimentally confirmed (Soares et al., 2009; Sasikumar et al., 2012). Beside the canonical role of eEF1A, delivering aa-tRNA to ribosomes during mRNA translation, it is thought to have several additional "moonlighting" functions. There are numerous reports describing the role of eEF1A in cytoskeleton organization, proteolysis, apoptosis, nuclear protein export, viral propagation and regulation of protein expression (Mateyak and Kinzy, 2010; Sasikumar et al., 2012; Schulz et al., 2014). It was proposed that eEF1A is a cytoplasmic component of the transcription dependent nuclear export of polyA-binding protein 1 (PABP1) and the von Hippel-Lindau (VHL) protein. A so called transcription-dependent nuclear export motif (TD-NEM) present in PAB1P1 and VHL was identified and shown to interact specifically with eEF1A (Khacho et al., 2008a,b).

In the present publication we provide evidence for protein–protein interaction between TIMAP and eEF1A1. Special importance of a TD-NEM-like motif present in TIMAP is clarified, and significance of the TIMAP-eEF1A1 interaction in endothelial cells was studied as well. It is shown that TIMAP-PP1 is involved in the regulation of the eEF1A1 phosphorylation level.

## 2. Materials and methods

### 2.1. Reagents

Materials were obtained from the following vendors: paraformaldehyde, dimethylsulfoxide, bovine serum albumin: Sigma (St Louis, MO). Dilutions of antibodies utilized is Western blots are given in parentheses. Rabbit polyclonal anti-PPP1R16B antibody (1:1000), anti-eEF1A1 antibody (1:1000): Abgent; Inc. (San Diego, CA); anti-EF1α antibody (1:1000): BD Transduction Laboratories (Heidelberg, Germany); anti-rabbit IgG HRP-linked and anti-mouse IgG HRP-linked secondary antibodies (1:5000), CD31(PECAM-1) antibody (1:1000), P-Thr specific antibody (1:2000): Cell Signaling Technology, Inc. (Beverly, MA); anti-β-tubulin (1:2000) and anti-PP1 delta antibodies (1:1000): Upstate Biotechnology (Lake Placid, NY); anti-lamin A/C (H-110) antibody (1:2000): Santa Cruz Biotechnology, Inc. (Santa Cruz, CA); mouse anti-c-myc antibody (1:2000), TO-PRO-3 Iodide, Lipofectamine 2000 and Lipofectamine RNAiMax: Life Technologies (Carlsbad, CA); Alexa 488-, Alexa 594-conjugated secondary antibodies (used in 1:300 dilution in immunofluorescent staining) and ProLong Gold Antifade medium: Molecular Probes (Eugene, OR), restriction enzymes, T4 DNA ligase: Thermo Scientific, Inc. (Vantaa, Finland); Protease Inhibitor Cocktail Set III: EMD Biosciences (San Diego, CA); pCMV-myc, pGEX-4T-2 and pGEX-4T-3 vectors: Clontech Laboratories, Inc. (Mountain View, CA). Substances for cell culturing were from PAA (Austria). All other chemicals were obtained from Sigma (St Louis, MO).

### 2.2. Cell cultures

Human Pulmonary Artery Endothelial Cells (HPAEC) (Lonza Group Ltd, Switzerland, catalogue No: CC-2530) and Bovine Pulmonary Artery Endothelial Cells (BPAEC) (American Type Tissue Culture Collection, Rockville, MD, culture line-CCL 209) were maintained as described before (Boratko et al., 2013).

### 2.3. SDS–PAGE and LC–MS/MS analysis

Proteins were resolved by SDS–PAGE and stained with Blue Silver solution. Liquid Chromatography with Tandem Mass Spectrometry Detection was performed by Dr. Tamás Janáky at the University of Szeged, Faculty of Medicine, Department of Medical Chemistry (Boratko et al., 2013).

### 2.4. Preparation of TIMAP and eEF1A1 constructs

The full length and several shorter recombinant TIMAP constructs were created as described earlier (Boratko et al., 2013). TIMAP 1–257 and TIMAP 1–257 G252A were derived from the bacterial full length TIMAP construct and cloned into pGEX-4T-3 vector using the following primer pairs. Forward: 5′-TGG GAT CCA TGG CCA GTC ACG TGG ACC-3′; reverse wt: 5′-TTA CTC GAG TCA CAC ATC CAC ACG CAC TCC ATG-3′; reverse G252A mutant: 5′-TTA CTC GAG TCA CAC ATC CAC ACG CAC TGC ATG-3′. Mammalian expression constructs for these truncated forms of TIMAP were amplified using the same reverse primer and the following forward primer: 5′-TTG AAT TCT TAT GGC CAG TCA CGT GGA C-3′. Human cDNA prepared from HPAEC cells by reverse transcription employing oligo dT primer was used for eEF1A1 amplification with the following primers and cloned into pGEX-4T-2 vector: forward: 5′-ATG GAT CCA TGG GAA AGG AGA AGA CCC A-3′; reverse: TTA CTC GAG TCA TTT AGC CTT CTG AGC TTT C-3′. All primers were synthesized by Integrated DNA Technologies (Coralville, IA). The DNA sequences of the constructs were confirmed by sequencing (Clinical Genomics Center and Department of Laboratory Medicine, University of Debrecen).

### 2.5. Bacterial expression and GST pull-down assay

*Escherichia coli* BL21 (DE3) transformed with pGEX-4T-3 containing glutathione S-transferase (GST), pGEX-4T-3 containing TIMAP mutants or pGEX-4T-2 containing eEF1A1 constructs were induced with 1 mM IPTG and grown at room temperature (RT) for 3 h. Harvested were sonicated in lysis buffer (50 mM Tris–HCl (pH 7.5), 0.1% Tween 20, 0.2% 2-mercaptoethanol, protease inhibitors) and proteins were isolated using glutathione Sepharose 4B (GE Healthcare, Piscataway, NJ) according to the manufacturer's protocol. For pGEX-4T-3 TIMAP 1–257 and pGEX-4T-3 TIMAP 1–257 G252A mutant contructs *E. coli* BL21 (DE3) cells were induced with 0.5 mM IPTG and grown at RT for 3 h. Harvested cells were sonicated in 0.2% sarcosyl containing lysis buffer. After centrifugation the supernatant of the samples were diluted in lysis buffer to contain 0.008% sarcosyl and proteins were isolated by affinity chromatography on glutathione Sepharose 4B. BPAEC grown in 100-mm culture flasks were washed twice with 1× ice-cold TBS, scraped, and lysed in 600 μl lysis buffer. The lysates were incubated with GST or GST-fused proteins coupled to glutathione Sepharose for 4 h at 4 °C. The beads were washed three times with 1× TBS then the GST fusion proteins were eluted by boiling the samples.

Download English Version:

https://daneshyari.com/en/article/8322474

Download Persian Version:

https://daneshyari.com/article/8322474

Daneshyari.com