We are IntechOpen, the world's leading publisher of Open Access books
Built by scientists, for scientists

4,800
Open access books available

122,000
International authors and editors

135M
Downloads

154
Countries delivered to

Our authors are among the
TOP 1%
most cited scientists

12.2%
Contributors from top 500 universities

WEB OF SCIENCE™

Selection of our books indexed in the Book Citation Index
in Web of Science™ Core Collection (BKCI)

Interested in publishing with us?
Contact book.department@intechopen.com

Numbers displayed above are based on latest data collected.
For more information visit www.intechopen.com

Chapter 5

Cellular Interaction of Human Eukaryotic Elongation Factor 1A Isoforms

Nunzia Migliaccio, Gennaro Sanità,
Immacolata Ruggiero, Nicola M. Martucci,
Carmen Sanges, Emilia Rippa,
Vincenzo Quagliariello, Ferdinando Papale,
Paolo Arcari and Annalisa Lamberti

Additional information is available at the end of the chapter

http://dx.doi.org/10.5772/intechopen.74733

Abstract

Besides its canonical role in protein synthesis, the eukaryotic translation elongation factor 1A (eEF1A) is also involved in many other cellular processes such as cell survival and apoptosis. We showed that eEF1A phosphorylation by C-Raf *in vitro* occurred only in the presence of eEF1A1 and eEF1A2, thus suggesting that both isoforms interacted in cancer cells (heterodimer formation). This hypothesis was recently investigated in COS-7 cells where fluorescent recombinant eEF1A isoforms colocalized at the level of cytoplasm with a FRET signal more intense at plasma membrane level. Here, we addressed our attention in highlighting and confirming this interaction in a different cell line, HEK 293, normally expressing eEF1A1 but lacking the eEF1A2 isoform. To this end, His-tagged eEF1A2 was expressed in HEK 293 cells and found to colocalize with endogenous eEF1A1 in the cytoplasm, also at the level of cellular membranes. Moreover, FRET analysis showed, in this case, the appearance of a stronger signal mainly at the level of the plasma membrane. These results confirmed what was previously observed in COS-7 cells and strongly reinforced the interaction among eEF1A isoforms. Moreover, the formation of eEF1A heterodimer in cancer cells could also be important for cytoskeleton rearrangements rather than for phosphorylation, most likely occurring during cell survival and apoptosis.

Keywords: eukaryotic translation elongation factor 1A (eEF1A), confocal microscopy, FRET, pull-down assay, immunoblotting

IntechOpen

© 2018 The Author(s). Licensee IntechOpen. This chapter is distributed under the terms of the Creative Commons Attribution License (http://creativecommons.org/licenses/by/3.0), which permits unrestricted use, distribution, and reproduction in any medium, provided the original work is properly cited.

1. Introduction

Eukaryotic elongation factor 1A (eEF1A) belongs to the family of GTP-binding proteins and it is the second most abundant protein in the cellular environment. It catalyzes the first step of the elongation cycle by promoting the GTP-dependent binding of aminoacyl-tRNA to the A-site of the ribosome [1–3]. eEF1A exists as two isoforms eEF1A1 and eEF1A2 [4], and in humans, they share almost identical amino acid sequences (92% sequence identity). eEF1A1 is ubiquitously present except in skeletal and cardiac muscle, while eEF1A2 expression is restricted in the brain, skeleton muscle, heart, and other cell types including large motor neurons, islet cells in the pancreas, and neuroendocrine cells in the gut [5], and it is currently found in all vertebrates [6]. Besides their role in polypeptide synthesis, paralogous human eEF1A1 and eEF1A2 act as "moonlighting" proteins [7] owing to several noncanonical functions such as cytoskeleton remodeling by binding and bundling filamentous actin [8, 9], apoptosis, nuclear transport, proteasome-mediated degradation of damaged proteins, heat shock, and transformation [10–12]. Overexpression of eEF1A1 or eEF1A2 in Hela cells led to increased cell growth [7], whereas the disruption of eEF1A1 resulted in actin cytoskeleton defects under basal conditions and in response to palmitate, thus suggesting that eEF1A1 mediates lipotoxic cell death, secondary to oxidative and ER stress, by regulating cytoskeletal changes critical for this process [13]. These findings highlighted that eEF1A1 was involved in both cell proliferation and apoptosis, though the relationship between eEF1A1 and apoptosis is still unclear. By contrast, eEF1A2 seems to play antiapoptotic properties in ovarian, breast, pancreatic, liver, and lung cancer [14]; however, this oncogenic potential deserves further investigation [15].

The possible interaction between eEF1A molecules was first characterized in *Tetrahymena* as eEF1A dimer was able to bundle actin filament [16]. Subsequently, the identification of dimeric eEF1A was also reported in both chicken and human B cell lines [17]. Recent investigations indicated that, compared to eEF1A2, eEF1A1 showed a higher property of self-association [18]. Moreover, under oxidant condition, eEF1A1 was able to form intermolecular disulfide bonds [19]. Recent findings showed that C-Raf kinase interacts *in vivo* with eEF1A during a survival response mediated by epidermal growth factor (EGF) following the treatment of human lung cancer cells with α-interferon (IFNα) [20]. Moreover, phosphorylation of *e*EF1A *in vitro* by C-Raf on S21 required the presence of both *e*EF1A isoforms, thus suggesting that the existence of an eEF1A1/eEF1A2 complex and the S21 phosphorylation represented a regulatory mechanism responsible for the switch from eEF1A canonical to noncanonic functions [21]. On the basis of these findings, we recently showed the possible direct interaction between the *e*EF1A isoforms by using fluorescence resonance energy transfer (FRET) [22]. Compared to our previous work, here we settled for a different experimental approach mainly based on pull-down, confocal microscopy, and FRET analysis based on IgG-FITC (donor)- and IgG-TRITC (acceptor)-conjugated antibodies in HEK 293 cells transfected with recombinant His-tagged eEF1A2 isoform.

2. Expression and interaction of eEF1A1 and eEF1A2 in HEK 293 cells

To assess the possible physiological interaction between eEF1A isoforms in a natural cellular environment, such as the cytoplasm of intact cells, human embryonic kidney 293 (HEK 293)

http://dx.doi.org/10.5772/intechopen.74733

cell line was used as an experimental system. This choice was derived from the finding that HEK 293 cells normally express substantial levels of eEF1A1 isoform, whereas the eEF1A2 isoform is absent.

2.1. Expression in HEK 293 of eEF1A1 and eEF1A2

First, the efficiency of pcDNA3.1-eEF1A2(His)$_6$ (gift from C. R. Knudsen, Aarhus, Denmark [23]) to transfect HEK 293 cells was evaluated. As reported in **Figure 1A**, compared to non-transfected HEK 293, cells transfected with recombinant eEF1A2 isoform showed an increase in the expression of the 54 kDa bands corresponding to the molecular weight of eEF1A. Subsequently, the expression level of eEF1A2 using a specific anti-eEF1A2 antibody (prepared as already reported [22]) was analyzed. As shown in **Figure 1B**, eEF1A2 isoform was revealed only in HEK 293 cells transfected with pcDNA3.1-eEF1A2(His)$_6$ and confirmed with the anti-His antibody (Merck, Germany) (**Figure 1C**).

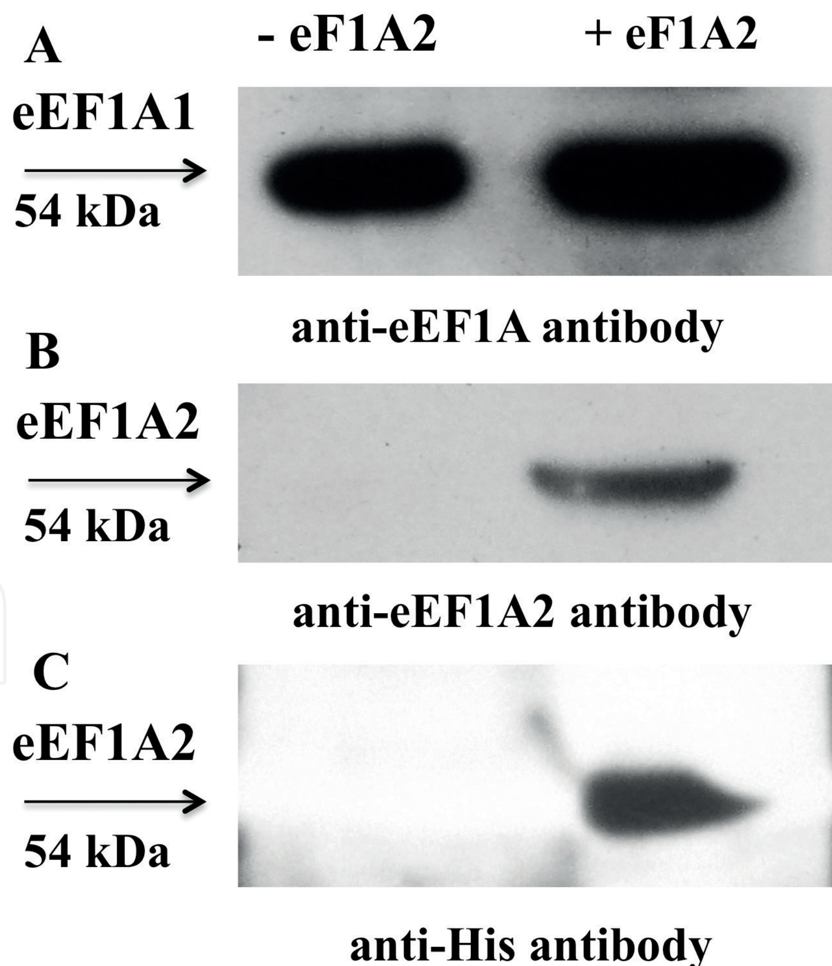

Figure 1. Expression of eEF1A isoforms in HEK 293 cells. HEK 293 cells were transfected with pcDNA3.1-eEF1A2(His)$_6$, and after 24 h from transfection, cell extracts were analyzed by Western blot using commercial mouse anti-eEF1A antibody (A), anti-eEF1A2 antibody (B), and rabbit anti-His antibody (C). Lanes: −eEF1A2, non-transfected HEK 293 cells; +eEF1A2, HEK 293 cells transfected with pcDNA3.1-eEF1A2(His)$_6$.

2.2. Both eEF1A1 and eEF1A2 immuno-interact after pull-down

The possible interaction between eEF1A isoforms was analyzed by pull-down experiment. To this purpose, GST-eEF1A1 (kindly supplied by C. Sanges, Wurzburg, Germany [21]) and pcDNA3.1-eEF1A2$(His)_6$ constructs were co-transfected in HEK 293 cells and, after 24 h from transfection, cell extracts were analyzed by Western blot following GST-agarose and Ni-NTA-agarose pull-down. As shown in **Figure 2**, compared to controls, GST pull-down of co-transfected cells showed the presence of a band of 54 kDa corresponding to the size of eEF1A2$(His)_6$ (**Figure 2A**, lane 2), whereas Ni-NTA pull-down showed the presence of a band of about 78 kDa corresponding to the size of the construct GST-eEF1A1 (**Figure 2B**, lane 2). **Figure 2B** (lane 3) also shows the presence of a band of about 26 kDa corresponding to the GST protein. This finding suggested that GST by itself somehow interacted with Ni-NTA matrix; thus, the result shown in line 2 could be partly due to an interaction of the GST moiety present in GST-eEF1A1 with Ni-NTA and not with eEF1A2. Therefore, to further confirm the interaction between eEF1A isoforms, a different approach was undertaken after transfection of HEK 293 cells with pcDNA3.1-eEF1A2$(His)_6$. In fact, as reported in **Figure 2C**, compared to cells transfected with pcDNA3.1 empty vector, cells transfected with eEF1A2$(His)_6$ showed, after Ni-NTA

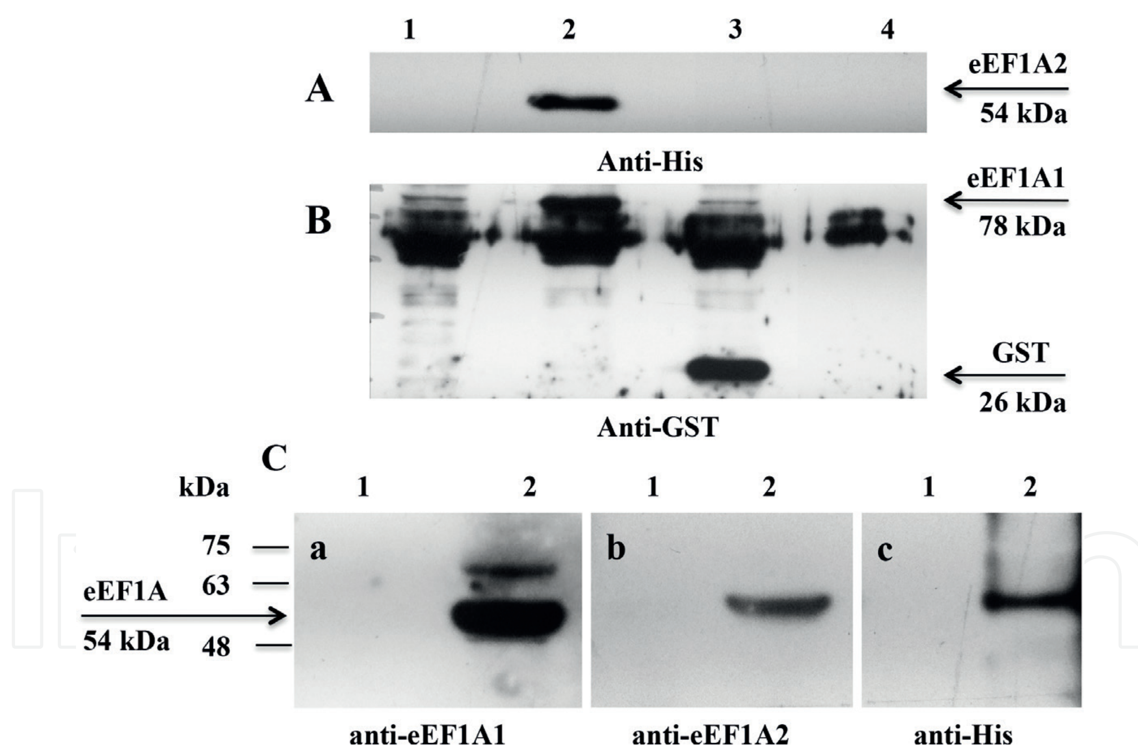

Figure 2. Co-transfection of GST-eEF1A1 and eEF1A2-His in HEK 293 cells. GST-eEF1A1 and pcDNA3.1-eEF1A2$(His)_6$ were cotransfected in HEK 293 7 cells. After 24 h, the cells were harvested, lysed, and analyzed after GST pull-down with antibody anti-His (A) and after Ni-NTA pull-down with anti-GST antibody (B). (A) Lanes: 1, non-transfected cells; 2, cells transfected with GST-eEF1A1 and pcDNA3.1-eEF1A2$(His)_6$; 3, cells transfected with GST and pcDNA3.1-eEF1A2$(His)_6$; 4, GST-agarose alone. (B) Lanes: 1, non-transfected cells; 2, cells transfected with GST-eEF1A1 and pcDNA3.1-eEF1A2$(His)_6$; 3, cells transfected with GST and pcDNA3.1-eEF1A2$(His)_6$; 4, Ni-NTA alone. (C) pcDNA3.1-eEF1A2$(His)_6$ was co-transfected in HEK 293 7 cells. After 24 h, the cells were harvested, lysed, and analyzed after Ni-NTA pull-down with anti-eEF1A1, anti-eEF1A2, and anti-His antibody. (a–c) Lanes: 1, cells transfected with empty vector; 2, cells transfected with pcDNA3.1-eEF1A2$(His)_6$.

http://dx.doi.org/10.5772/intechopen.74733

pull-down of cell extracts, the presence of a band of 54 kDa that was recognized by the specific anti-eEF1A1 (prepared as already reported [22]) (**Figure 2C**-a, lane 2) and anti-eEF1A2 (**Figure 2C**-b, lane 2) antibodies, the latter confirmed also with anti-His antibody (**Figure 2C**-c, lane 2).

2.3. Both eEF1A1 and eEF1A2 colocalize in HEK 293 cells

The intracellular colocalization of eEF1A1 and eEF1A2 was first analyzed by confocal microscopy. As shown in **Figure 3**, HEK 293 cells after 48 h from transfection with pcDNA3.1-eEF1A2(His)$_6$ construct revealed that both endogenous eEF1A (**Figure 3A**) and transfected eEF1A2(His)$_6$ (**Figure 3B**) shared a cytoplasmic localization. The superimposition of the two panels (merged image, **Figure 3D**) showed that both eEF1A isoforms exhibited a cytoplasmic colocalization with specific signals more intense at the level of the plasma membrane.

2.4. FRET analysis showed that both eEF1A1 and eEF1A2 interact in HEK 293 cells

The interaction between endogenous eEF1A and transfected eEF1A2(His)$_6$ was further investigated by sensitized emission FRET method. FRET effect was performed by confocal microscope

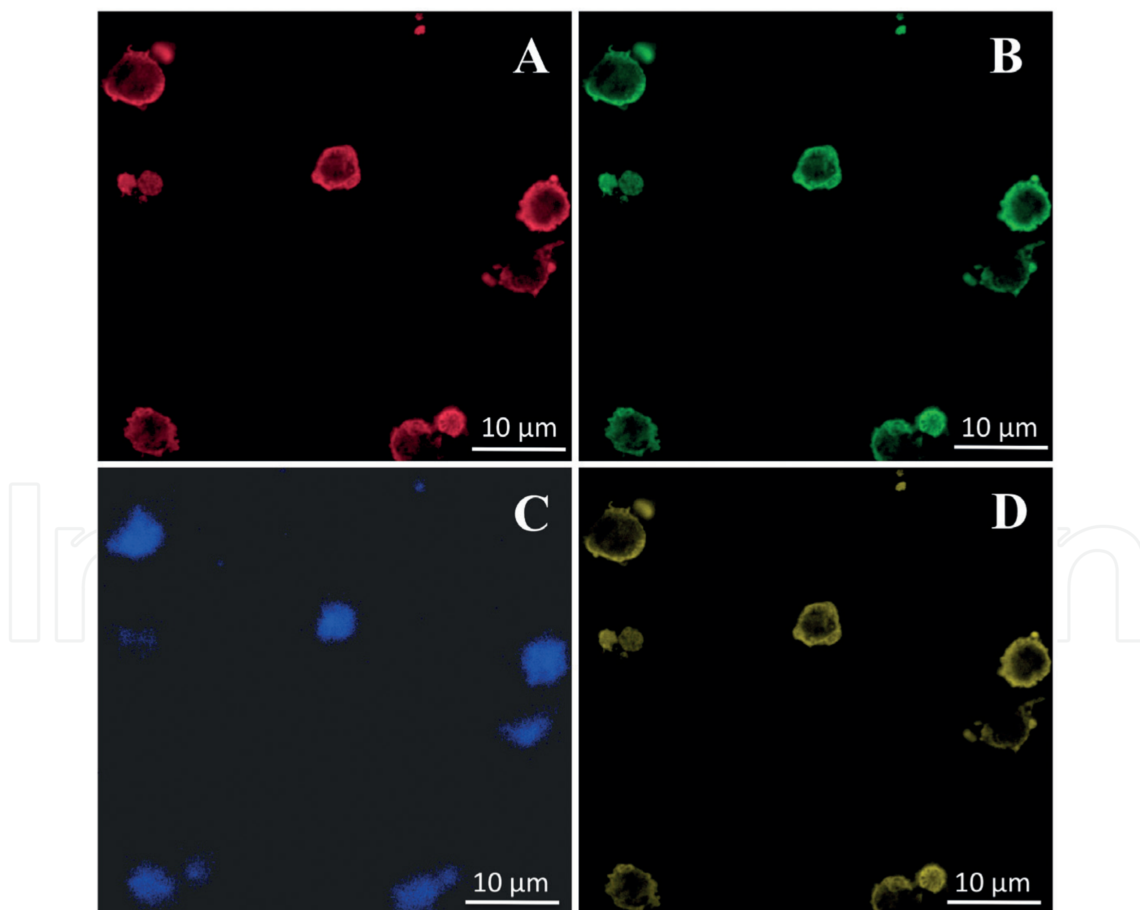

Figure 3. Colocalization of eEF1A1 and eEF1A2(His)$_6$ in HEK 293 cells. HEK 293 cells were transfected with pcDNA3.1-eEF1A2(His)$_6$, and after 48 h from transfection, cells were analyzed by confocal microscopy. (A) eEF1A1, (B) eEF1A2, (C) nuclear staining, and (D) merged images.

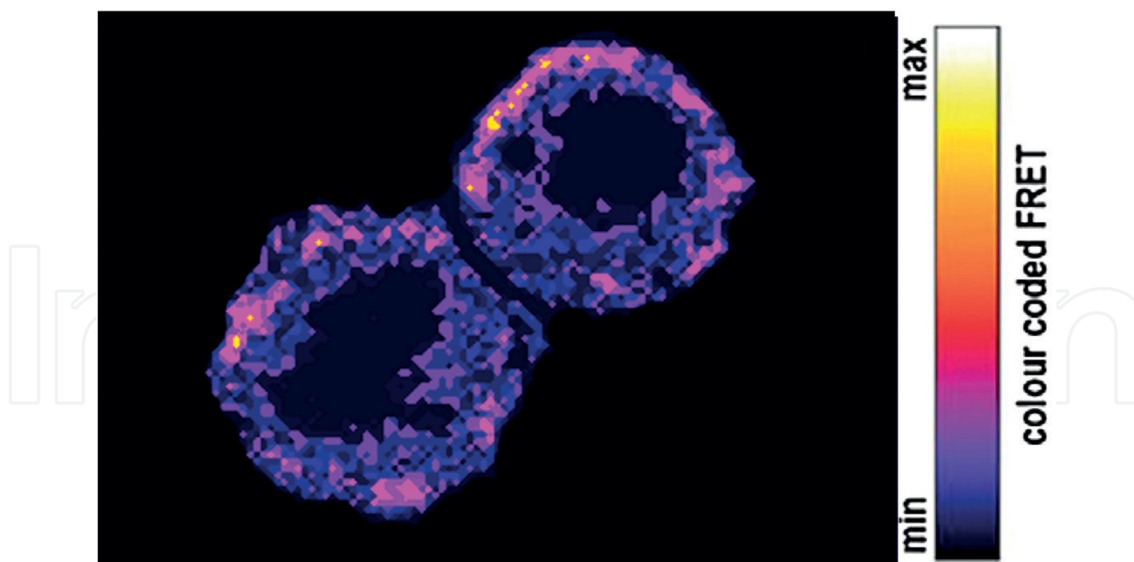

Figure 4. FRET analysis of the interaction between eEF1A1 and eEF1A2. Representative pseudocolor images of cells labeled with rabbit anti-eEF1A1 and mouse anti-His primary antibodies followed by FITC and TRITC secondary-labeled antibodies.

that allowed discriminate proteins that colocalize in the same cellular compartment from those that are instead involved in specific molecular interactions. FRET effects were calculated using ImageJ plug-in software [24]. **Figure 4** shows the representation of the FRET effects where the blue color is indicated at low signal, whereas yellow-white color designated a high signal. The images clearly showed the interaction between eEF1A1 and transfected eEF1A2(His)$_6$ within the cytoplasm with specific signals more intense especially at the level of the plasma membrane.

3. Discussion

FRET is a powerful technique suitable for studying *in situ* interactions between biological molecules in cellular environments [25]. FRET can be assessed from the transfer of energy from one fluorescent molecule (donor) to another fluorescent molecule (acceptor). This process occurs optimally only if the two molecules are properly oriented and reasonably at a narrow distance (usually 1–10 nm) [26]. By this technique, the interaction between eEF1A1 and eEF1A2 in order to reinforce our hypothesis on the formation of an eEF1A1-eEF1A2 heterodimer [21] was highlighted. In a different approach, we afforded this aspect by expressing chimeric eEF1As fused to CFP and YFP as donor and acceptor (CFP-eEF1A1 and YFP-eEF1A2) in COS-7 cells, respectively [22]. However, some criticisms emerged that could have affected the FRET results such as (1) the possible interaction of the expressed chimeric proteins with endogenous enzymes, (2) self-association between eEF1A molecules (i.e., homodimer formation), and (3) the overexpression in COS-7 cells of both constructs that could have generated an art factual FRET signal mainly at the level of plasma membrane. Therefore, to overcome these concerns and to confirm that both eEF1A isoforms interact in the cellular environment, we used a different approach based on the use of IgG-FITC (donor)- and IgG-TRITC (acceptor)-conjugated antibodies. To this end, HEK 293 cell line, lacking the expression of eEF1A2 isoform, was transfected with

http://dx.doi.org/10.5772/intechopen.74733

pcDNA3.1-eEF1A2(His)$_6$, and the interaction of the recombinant eEF1A2(His)$_6$ with endogenous eEF1A1 was assessed by pull-down, confocal microscopy and FRET analysis. The results obtained showed that the endogenous eEF1A1 and the expressed eEF1A2 interacted in HEK 293 cells at the level of both cytoplasm and plasma membrane. Moreover, the FRET image highlighted a more intense signal at the level of the plasma membrane. These data confirmed those reported in our previous work [22], thus strongly confirming the association in the cells of eEF1A isoforms.

The homodimer association of eEF1As has recently emerged from the crystallization of rabbit eEF1A2 [27] or as proposed in *Tetrahymena*, in order to explain actin bundling essential for the regulation of actin cytoskeleton and cell morphology during several cellular processes [16]. The possible association between eEF1A isoforms was instead proposed by Sanges et al. [21] in studying the control of eEF1A function in cancer cells *via* phosphorylation and by Lee et al. [28] in studying the interaction of eEF1A2 with the tumor suppressor protein p16^{INK4a}. Since eEF1A1 and eEF1A2 display a very high amino acid sequence identity (above 97%), the overall structures appear quite similar, as can be predicted by bioinformatic analysis at the GRAMM-X docking Web Server v.1.2.0 [29, 30], using rabbit eEF1A2 (PDB 4C0S chain A) as template [27]. These considerations suggest that both eEF1A1 and eEF1A2 complexes are present in the cells either as homodimer or as heterodimer. These complexes are most likely associated with regulatory noncanonical functions of eEF1As.

4. Conclusions

Because eEF1A dimers are involved in actin bundling [31, 32], it emerges that the fraction of eEF1A as dimer is mostly involved in the actin cytoskeleton rearrangement. Therefore, the cellular distribution of eEF1A molecules between monomeric and dimeric form regulates the functional role of eEF1A in translation or in actin bundling. Because actin chains and translational system coexist in the cells and maybe also functionally dependent [33, 34], the transition "monomer-dimer-monomer" of eEF1A should be relatively easy depending on the cell conditions [35]. This interconversion may be regulated by the reversible posttranslational modifications of eEF1A [36] and its interactions with the protein partners such as Raf kinases [20, 21]. Therefore, it is possible that in cells coexpressing both isoforms, like cancer cells, eEF1A heterodimer formation could also be important for cytoskeleton rearrangements rather than for some phosphorylation catalysis most likely occurring during cell survival and apoptosis [20, 21].

5. Materials and methods

5.1. Cell culture and transfection

HEK 293 cells, obtained from the American Type Tissue Collection (Rockville, MD, USA), were grown at 37°C in a 5% CO_2 atmosphere in Dulbecco's modified Eagle medium (DMEM) (Gibco, Monza, Italy) supplemented with 10% heat-inactivated FBS (GIBCO), 100 U/ml penicillin, 100 mg/ml streptomycin, and 1% L-glutamine.

For Western blot analysis, cells (300 × 10^3/well) were transfected with GST-eEF1A1 (1 μg), pcDNA3.1-eEF1A2(His)$_6$ (1 μg) and pcDNA3.1 (3 μg) as control using Lipofectamine 2000 or K2. Twenty-four hours after transfection, cells were collected and the corresponding extract analyzed with mouse monoclonal anti-eEF1A antibody.

For confocal microscopy and FRET analysis, cells (10 × 10^3) were layered on 10-mm glass coverslips, grown at confluence and then transfected with pcDNA3.1-eEF1A2(His)$_6$ (1 μg) or with pcDNA3.1 (1 μg) as controls. Cells were analyzed after 24 h of incubation.

5.2. Cytosolic extracts, pull-down assay, and Western blot

After growth, HEK 293 were scraped, washed twice in PBS, resuspended for 30 min on ice in 20–40 μl of lysis buffer (50 mM Tris-HCl pH 7.4, 1% NP40, 0.25% sodium deoxycholate, 150 mM NaCl, 1 mg/ml aprotinin, leupeptin, pepstatin, 1 mM Na3VO4, 1 mM NaF), and then centrifuged at 14,000 × *g* for 20 min at 4°C.

Pull-down assay for GST-eEF1A1 or eEF1A2(His)$_6$ was carried out using GST-sepharose (Amersham, Milan, Italy) or Ni-NTA agarose (Qiagen, Milan, Italy), respectively. In detail, 500 μg of cell extracts was incubated with pre-equilibrated resin (about 150 μl slurry/1 mg protein extract) for 2 h at room temperature (RT) or ON at 4°C, respectively. Subsequently, for GST pull-down, the resin was washed two times (centrifugation for 2 min at 2000 r.p.m. 4°C) with 1 ml of 1× phosphate-buffered saline (PBS), whereas for Ni-NTA pull-down, the resin was washed two times with 50 mM NaH_2PO_4, 300 mM NaCl, and 20 mM imidazole, to reduce nonspecific bound proteins, 0.05% Tween 20, pH 8.0. Successively, the samples were resuspended in 30 μl of 4× Laemmli loading buffer, heated to 95°C for 15 min and subjected to Western blot analysis.

Protein concentration was determined by a modified Bradford method, using the Bio-Rad protein assay and compared with bovine serum albumin (BSA) standard curve. Blots were developed using enhanced chemiluminescence detection (SuperSignal West Pico, Pierce, Milan, Italy). All films were scanned using Adobe Photoshop Software (San Jose, CA, USA).

5.3. Confocal laser scanning microscopy

Human embryonic kidney cells (HEK 293 Cell line) were treated for 20 min with glutaraldehyde 2.5% in PBS, washed three times with PBS, permeabilized for 10 min with 0.1% Triton-X100 and finally washed in PBS. Cells were then blocked for 20 min with 1% BSA in PBS, and after apposite washes, cells were incubated with rabbit anti-EF1A1 antibody (GenScript, Piscataway, NJ, USA) and mouse anti-His polyclonal antibody (Santa Cruz Biotechnology, Santa Cruz, CA, USA) diluted 1:300 in 1% BSA for 1 h. After washing three times with PBS, cells were incubated for 1 h with the appropriate secondary antibodies conjugated to fluorochromes and diluted 1:1000 in 1% BSA. Incubation with TOPRO 3-Iodide (Invitrogen Molecular Probes Eugene, OR, USA) diluted 1/1000 in BSA 1% was done for staining of the nucleus. After this, cells were washed properly with PBS and then observed with a Nikon Confocal Microscope C1 equipped with an EZ-C1 Software for data acquisition by using 60× oil immersion objective.

http://dx.doi.org/10.5772/intechopen.74733

5.4. FRET analysis

HEK 293 cells (7×10^3 cells/cm^2) were grown for 24 h on glass coverslips under standard conditions (37°C, 5% CO_2). Cells were then rinsed with PBS, fixed for 10 min with formaldehyde (3.7% in PBS), permeabilized for 10 min with Triton X-100 (0.1% in PBS), and blocked for 20 min in bovine serum albumin (BSA) (1% in PBS). Subsequently, each sample was incubated for 1 h with 5 μg/ml of mouse anti-His and 5 μg/ml of human anti-eEF1A1 antibodies. Following PBS washes, cells were treated for 1 h with goat anti-mouse IgG FITC-conjugated antibody (donor) (Santa Cruz Biotechnology, Santa Cruz, CA, USA) (1 μg/ml) and with goat anti-rabbit IgG-TRITC-conjugated antibody (acceptor) (Santa Cruz Biotechnology, Santa Cruz, CA, USA) (10 μg/ml), Finally, after 3× washes in PBS, confocal images were collected using a Nikon Confocal Microscope C1 furnished with EZ-C1 software. FRET analysis was carried out as already reported [24]. "FRET" images give the calculated amount of FRET for each pixel in the merged images. The ImageJ plug-in color codes the relative FRET efficiency, which is reported by the displayed color bar, on the right of the images.

Acknowledgements

This work was supported by funds from Programmi di Ricerca Scientifica di Rilevante Interesse Nazionale (2012CK5RPF_004), PON Ricerca e Competitività 2007-2013 (PON01_02782), and POR Campania FSE 2007-2013, Project CRÈME.

Conflicts of interest

The authors declare that there is no conflict of interest.

Disclosure statement

Nothing to declare.

Author contributions

NM, IR, and NMM were involved in WB analysis and cell extract preparation; GS and CS were involved in cell transfection; ER was in charge of tissue culture; VQ and FP performed confocal and FRET analysis; PA and AL were involved in the reading and approval of the manuscript.

IntechOpen

Abbreviations

CFP	cyan fluorescent protein
DMEM	Dulbecco's modified Eagle medium
eEF1A	eukaryotic elongation translation factor 1A
EGF	epidermal growth factor
FBS	fetal bovine serum
FRET	fluorescence resonance energy transfer
GST	glutathione S-transferase
Ni-NTA	nickel-nitrilotriacetic acid
PBS	phosphate-buffered saline
TRITC	tetramethylrhodamine
YFP	yellow fluorescent protein

Author details

Nunzia Migliaccio[1], Gennaro Sanità[1], Immacolata Ruggiero[1], Nicola M. Martucci[1], Carmen Sanges[2], Emilia Rippa[1], Vincenzo Quagliariello[3], Ferdinando Papale[4], Paolo Arcari[1,5]* and Annalisa Lamberti[1]

*Address all correspondence to: arcari@unina.it

1 Department of Molecular Medicine and Medical Biotechnology, University of Naples Federico II, Naples, Italy

2 ERT, eResearchTechnology GmbH, Estenfeld, Germany

3 Department of Abdominal Oncology, National Cancer Institute "G. Pascale", Naples, Italy

4 Department of Thoracic and Cardio-respiratory Sciences, University of Campania L. Vanvitelli, Naples, Italy

5 CEINGE, Advanced Biotechnology Scarl, Naples, Italy

References

[1] Kaziro Y, Itoh H, Kozasa T, Nakafuku M, Satoh T. Structure and function of signal-transducing GTP-binding proteins. Annual Review of Biochemistry. 1991;**60**:349-400. DOI: 10.1146/annurev.bi.60.070191.002025

[2] Klink F. In: Woese CR, Wolfe R, editors. The Bacteria. Vol. 8. London: Academic Press; 1985. pp. 379-410

[3] Moldave K. Eukaryotic protein synthesis. Annual Review of Biochemistry. 1985;**54**: 1109-1149. DOI: 10.1146/annurev.bi.54.070185.005333

[4] Lund A, Knudsen SM, Vissing H, Clark B, Tommerup N. Assignment of human elongation factor 1alpha genes: EEF1A maps to chromosome 6q14 and EEF1A2 to 20q13.3. Genomics. 1996;**36**:359-361. DOI: 10.1006/geno.1996.0475

[5] Newbery HJ, Loh DH, O'Donoghue JE, Tomlinson VAL, Chau YY, Boyd JA, Bergmann JH, Brownstein D, Abbott CM. Translation elongation factor eEF1A2 is essential for post-weaning survival in mice. The Journal of Biological Chemistry. 2007;**282**:28951-28959. DOI: 10.1074/jbc.M703962200

[6] Newbery HJ, Stancheva I, Zimmerman LB, Abbott CM. Evolutionary importance of translation elongation factor eEF1A variant switching: EEF1A1 downregulation in muscle is conserved in Xenopus but is controlled at a posttranscriptional level. Biochemical and Biophysical Research Communications. 2011;**411**:19-24. DOI: 10.1016/j.bbrc.2011.06.062

[7] Mateyak MK, Kinzy TG. eEF1A: Thinking outside the ribosome. The Journal of Biological Chemistry. 2010;**285**:21209-21213. DOI: 10.1074/jbc.R110.113795

[8] Murray JW, Edmonds BT, Liu G, Condeelis J. Bundling of actin filaments by elongation factor 1α inhibits polymerization at filament ends. The Journal of Cell Biology. 1996;**135**:1309-1321. DOI: 10.1083/jcb.135.5.1309

[9] Gross SR, Kinzy TG. Translation elongation factor 1A is essential for regulation of the actin cytoskeleton and cell morphology. Nature Structural & Molecular Biology. 2005;**12**:772-778. DOI: 10.1038/nsmb979

[10] Ejiri S. Moonlighting functions of polypeptide elongation factor 1: From actin bundling to zinc finger protein R1-associated nuclear localization. Bioscience, Biotechnology, and Biochemistry. 2002;**66**:1-21. DOI: 10.1271/bbb.66.1

[11] Chuang SM, Chen L, Lambertson D, Anand M, Kinzy TG, Madura K. Proteasome-mediated degradation of cotranslationally damaged proteins involves translation elongation factor 1A. Molecular and Cellular Biology. 2005;**25**:403-413. DOI: 10.1128/MCB.25.1.403-413.2005

[12] Hotokezaka Y, Tobben U, Hotokezaka H, Van Leyen K, Beatrix B, Smith DH, Nakamura T, Wiedmann M. Interaction of the eukaryotic elongation Factor 1A with newly synthesized polypeptides. The Journal of Biological Chemistry. 2002;**277**:18545-18551. DOI: 10.1074/jbc.M201022200

[13] Borradaile NM, Buhman KK, Listenberger LL, Magee CJ, Morimoto ET, Ory DS, Schaffer JE. A critical role for eukaryotic elongation factor 1A-1 in lipotoxic cell death. Molecular Biology of the Cell. 2006;**17**:770-778. DOI: 10.1091/mbc.E05-08-0742

[14] Lee MH, Surh YJ. eEF1A2 as a putative oncogene. Annals of the New York Academy of Sciences. 2009;**1171**:87-93. DOI: 10.1111/j.1749-6632.2009.04909.x

[15] Tomlinson VA, Newbery HJ, Wray NR, Jackson J, Larionov A, Miller WR, Dixon JM, Abbott CM. Translation elongation factor eEF1A2 is a potential oncoprotein that is overexpressed in two-thirds of breast tumors. BMC Cancer. 2005;**5**:113. DOI: 10.1186/1471-2407-5-113

[16] Bunai F, Ando K, Ueno H, Numata O. Tetrahymena eukaryotic translation elongation factor 1A (eEF1A) bundles filamentous actin through dimer formation. Journal of Biochemistry. 2006;**140**:393-399. DOI: 10.1093/jb/mvj169

[17] HaÅNsler J, Rada C, Neuberger MS. The cytoplasmic AID complex. Seminars in Immunology. 2012;**24**:273-280. DOI: 10.1016/j.smim.2012.05.004

[18] Timchenko AA, Novosylna OV, Prituzhalov EA, Kihara H, El'skaya AV, Negrutskii BS, Serdyuk IN. Different oligomeric properties and stability of highly homologous A1 and proto-oncogenic A2 variants of mammalian translation elongation factor eEF1. Biochemistry. 2013;**52**:5345-5353. DOI: 10.1021/bi400400r

[19] Cumming RC, Andon NL, Haynes PA, Park M, Fischer WH, Schubert D. Protein disulfide bond formation in the cytoplasm during oxidative stress. The Journal of Biological Chemistry. 2004;**279**:21749-21758. DOI: 10.1074/jbc.M312267200

[20] Lamberti A, Longo O, Marra M, Tagliaferri P, Bismuto E, Fiengo A, Viscomi C, Budillon A, Rapp UR, Wang E, Venuta S, Abbruzzese A, Arcari P, Caraglia M. C-RAF antagonizes apoptosis induced by IFN-alpha in human lung cancer cells by phosphorylation and increase of the intracellular content of elongation factor 1A. Cell Death and Differentiation. 2007;**14**:952-962. DOI: 10.1038/sj.cdd.4402102

[21] Sanges C, Scheuermann C, Zahedi RP, Sickmann A, Lamberti A, Migliaccio N, Baljuls A, Marra M, Zappavigna S, Rapp U, Abbruzzese A, Caraglia M, Arcari P. Raf kinases mediate the phosphorylation of eukaryotic translation elongation factor 1A and regulate its stability in eukaryotic cells. Cell Death & Disease. 2012;**3**:e276. DOI: 10.1038/cddis.2012.16

[22] Migliaccio N, Ruggiero I, Martucci NM, Sanges C, Arbucci S, Tatè R, Rippa E, Arcari P, Lamberti A. New insights on the interaction between the isoforms 1 and 2 of human translation elongation factor 1A. Biochimie. 2015;**118**:1-7. DOI: 10.1016/j.biochi.2015.07.021

[23] Kahns S, Lund A, Kristensen P, Knudsen CR, Clark BF, Cavallius J, Merrick WC. The elongation factor 1 A^{-2} isoform from rabbit: Cloning of the cDNA and characterization of the protein. Nucleic Acids Research. 1998;**26**:1884-1890. DOI: 10.1093/nar/26.8.1884

[24] Hachet-Haas M, Converset N, Marchal O, Matthes H, Gioria S, Galzi JL, Lecat S. FRET and colocalization analyzer method to validate measurement of sensitized emission FRET acquired by confocal microscopy and available as an ImageJ plug-in. Microscopy Research and Technique. 2006;**69**:e941-e956. DOI: 10.1002/jemt.20376

[25] Sekar RB, Periasamy A. Fluorescence resonance energy transfer (FRET) microscopy imaging of live cell protein localizations. The Journal of Cell Biology. 2003;**160**:629-633. DOI: 10.1083/jcb.200210140

[26] Konig P, Krasteva G, Tag C, Konig IR, Arens C, Kummer W. FRET-CLSM and double-labeling indirect immunofluorescence to detect close association of proteins in tissue sections. Laboratory Investigation. 2006;**86**:853-864. DOI: 10.1038/labinvest.3700443

[27] Crepin T, Shalak VF, Yaremchuk AD, Vlasenko DO, McCarthy A, Negrutskii BS, Tukalo MA, El'skaya AV. Mammalian translation elongation factor eEF1A2:X-ray structure and new features of GDP/GTP exchange mechanism in higher eukaryotes. Nucleic Acids Research. 2014;**42**:12939-12948. DOI: 10.1093/nar/gku974

[28] Lee MH, Choi BY, Cho YY, Lee SY, Huang Z, Kundu JK, Kim MO, Kim DJ, Bode AM, Surh YJ, Dong Z. Tumor suppressor p16INK4a inhibits cancer cell growth by downregulating eEF1A2 through a direct interaction. Journal of Cell Science. 2013;**126**:3796. DOI: 10.1242/jcs.113613

[29] Tovchigrechko A, Vakser IA. Development and testing of an automated approach to protein docking. Proteins. 2005;**60**:296-301. DOI: 10.1002/prot.20573

[30] Tovchigrechko A, Vakser IA. GRAMM-X public web server for protein-protein docking. Nucleic Acids Research. 2006;**34**:W310-W314. DOI: 10.1093/nar/gkl206

[31] Stevenson RP, Veltman D, Machesky LM. Actin-bundling proteins in cancer progression at a glance. Journal of Cell Science. 2012;**125**:1073-1079. DOI: 10.1242/jcs.093799

[32] Tolbert CE, Burridge K, Campbell SL. Vinculin regulation of F-actin bundle formation: What does it mean for the cell? Cell Adhesion & Migration. 2013;**7**:219-225. DOI: 10.4161/cam.23184

[33] Stapulionis R, Kolli S, Deutscher MP. Efficient mammalian protein synthesis requires an intact F-actin system. The Journal of Biological Chemistry. 1997;**272**:24980-24986. DOI: 10.1074/jbc.272.40.24980

[34] Perez WB, Kinzy TG. Translation elongation factor 1A mutants with altered actin bundling activity show reduced aminoacyl-tRNA binding and alter initiation via eIF2alpha phosphorylation. The Journal of Biological Chemistry. 2014;**289**:20928-20938. DOI: 10.1074/jbc.M114.570077

[35] Vlasenko DO, Novosylna OV, Negrutskii BS, El'skaya AV. Truncation of the A,A/,A0 helices segment impairs the actin bundling activity of mammalian eEF1A1. FEBS Letters. 2015;**589**:1187-1193. DOI: 10.1016/j.febslet.2015.03.030

[36] Negrutskii B, Vlasenko D, El'skaya A. From global phosphoproteomics to individual proteins: The case of translation elongation factor eEF1A. Expert Review of Proteomics. 2012;**9**:71-83. DOI: 10.1586/epr.11.71

IntechOpen

IntechOpen