Analysis of $eEF1B\gamma$ interactome in the nuclear fraction of $A549$ human lung adenocarcinoma cells

L. M. Kapustian, I. L. Lysetsky, T. V. Bondarchuk, O. V. Novosylina, B. S. Negrutskii

Institute of Molecular Biology and Genetics, NAS of Ukraine
150, Akademika Zabolotnoho Str., Kyiv, Ukraine, 03143
negrutskii@imbg.org.ua

Aim. To study the translation elongation factor $eEF1B\gamma$ in the nucleus of lung carcinoma cells. Methods. The protein partners of $eEF1B\gamma$ in the nuclear fraction of A549 cells were identified by co-immunoprecipitation (co-IP) and liquid chromatography-tandem mass spectrometry (LC-MS/MS). The protein interaction network for nuclear $eEF1B\gamma$ was determined by Cytoscape 3.2.0 program using the MCODE plugin. Additional analysis of the $eEF1B\gamma$ partners was conducted by Map of the cell database. Results. 234 proteins interacting with $eEF1B\gamma$ in the nuclear fraction of A549 cells were identified. Possible functional networks involving these contacts were analyzed by two bioinformatic approaches. Conclusions. Splicing of pre-mRNA and regulation of mRNA stability are assumed to be the main processes in which nuclear $eEF1B\gamma$ can be involved. We hypothesize that a portion of $eEF1B\gamma$ leaves the cytoplasm-localized $EF1B$ complex during carcinogenesis and enters the nucleus to regulate certain mRNAs by affecting the splicing of their pre-mRNA and/or stability of mRNA.

Keywords: $eEF1B\gamma$, protein-protein interactions, nucleus, A549 cell

Introduction

The high molecular weight complex of translation elongation factors, which is called $eEF1B$, provides efficient GDP/GTP exchange in the molecule of another translation factor, $eEF1A$ [1]. $eEF1A$*GTP binds aminoacyl-tRNA of any specificity and brings it to the pre-A site of the 80S ribosome [2, 3]. Hydrolysis of GTP finalizes the step of codon-anticodon recognition permitting an aminoacyl-tRNA to be fully established in the A site. After some pause, length of which may depend on tRNA specificity [4], $eEF1A$*GDP leaves the ribosome. In principle, the exchange of GDP for GTP in $eEF1A$ can occur spontaneously as the cellular concentration of GTP is much higher than that of GDP. Nevertheless, the $eEF1B$ complex accelerates the nucleotide exchange process leading to the formation of a new $eEF1A$*GTP*aminoacyl-tRNA complex [5].
eEF1B comprises eEF1Bα, eEF1Bβ and eEF1Bγ subunits. eEF1Bγ is the only subunit of eEF1B which does not catalyze nucleotide exchange in eEF1A. Instead, it is believed to serve as “glue” holding all subunits of eEF1B together [6]. However, the existence of free eEF1Bγ was detected in human cancer tissues. Moreover, cancer cells showed a sign of nuclear localization of eEF1Bγ [7, 8]. As there is a general belief that protein synthesis occurs exclusively in cytoplasm [9], the cyto-nuclear transfer of eEF1Bγ implies some non-translational role of the subunit.

Here, in an attempt to clarify novel functions of the eEF1Bγ subunit in cancer cells, we carried out bioinformatic and experimental analysis of the protein partners of this subunit in the nuclear fraction of human lung cancer cells A549 and envisaged non-translational processes and networks involving eEF1Bγ.

As a result, we propose the splicing of pre-mRNA and regulation of mRNA stability as two main processes in which nuclear eEF1Bγ can be involved.

**Materials and Methods**

**Preparation of nuclear fraction of human lung cells**

Human lung cancer cells A549 were grown up to $7.5 \times 10^6$ cells/ml and harvested with Trypsin-EDTA. The nuclear fraction was isolated as in [10]. Briefly, cells were lysed in 1.5 volume of the buffer containing 10 mM HEPES pH7.9; 1.5 mM MgCl$_2$; 10 mM KCl; 0.5 % NP-40; 0.2 mM PMSF; 0.5 mM DTT for 20 min on ice. The centrifugation was performed (400g, 10 min) and the precipitate was resuspended in 4.5 volumes of the buffer (10 mM HEPES, 0.25 mM sucrose, 1.5 mM MgCl$_2$, 10 mM KCl, 0.1 % NP-40, 0.5 mM DTT, 0.2 mM protease inhibitor PMSF) on ice for 10 min to provide the protein extraction. The sucrose cushion (2M) centrifugation was performed at 400g for 10 min. Nuclei were re-suspended in the lysis buffer and centrifuged at 1500g for 10 min. The procedure was double-repeated. The nuclear pellet was re-suspended in a half of initial volume of the nucleus lysis buffer (20 mM HEPES pH 7.9, 25 % glycerol, 0.42 M NaCl, 1.5 mM MgCl$_2$, 0.2 mM DTT, 0.2 mM EDTA, 0.2 mM PMSF), incubated on ice for 30 min and centrifuged at 16000g for 30 min. The supernatant was used as a protein the nuclear fraction. The absence of the cytoplasmic fraction admixture was controlled by Western blot with anti-Tubulin antibodies (Abnova, Taiwan) [8].

**Co-immunoprecipitation**

The nuclear extract was pre-cleared with Protein G Sepharose (Sigma, USA) for 1 hour at 4 °C. Anti-eEF1Bγ antibodies (Abnova, Taiwan) (1.5 μg of antibodies per 1 mg of total protein) were added to pre-cleared lysate for overnight incubation at 4 °C. After addition of the Protein G Sepharose slurry the incubation persisted for 2 hours at 4 °C with continuous shaking. The eluted proteins were loaded on 12 % PAGE [11]. The protein bands of interest were cut and processed for mass-spectrometry analysis (LC-MS/MS).

**Mass-spectrometry LC-MS/MS**

The nuclear extract incubated with plain G-Sepharose was used as a control of nonspecific binding. The electrophoretic bands that were not present in the control or were much
more extensive than in the control were cut and processed for mass spectrometry analysis at the Mass Spectrometry Laboratory of the Institute of Biochemistry and Biophysics (Warsaw, Poland) as described before [12].

**Bioinformatic analysis**

Cytoscape 3.2.0 Program [13] interaction database BIOGRID was supplemented with newly identified nuclear protein partners of eEF1Bγ and analyzed by MCODE plugin which finds highly interconnected regions (clusters) in any network loaded into Cytoscape. These clusters have been shown to represent protein complexes and/or parts of pathways [14]. For the sake of clarity, the known protein partners of eEF1Bγ: eEF1A1, eEF1A2 and UBC (polyubiquitin-C) were excluded from the database [12]. Also, we limited analysis by the first (direct) partners of the eEF1Bγ partners. MCODE analysis was performed on the hybrid supercomputer “SCIT-4” of the Glushkov Institute of Cybernetics (GIC) of National Academy of Sciences of Ukraine (http://icybcluster.org.ua).

Analysis of the nuclear protein partners which are co-fractionated with eEF1Bγ was done using the Mapofthecell program (http://www.mapofthecell.org). All protein partners of the nucleus-localized eEF1Bγ identified by co-precipitation studies were tested for a possibility of their co-fractionation with eEF1Bγ as described in [10] for eEF1Bβ.

**Results and Discussion**

Having established a procedure for the cytonucleo fractionation of human lung carcinoma A549 cells [10] we isolated the nuclear fraction and conducted co-immunoprecipitation of the eEF1Bγ protein partners using anti-eEF1Bγ antibodies. Subsequent LC-MS/MS identification of precipitated proteins has delivered 234 interacting partners of eEF1Bγ.

These partners were used for further analysis by the Cytoscape program as described before [15]. Based on published data the Cytoscape protein interaction network of eEF1Bγ encompassed 11 proteins (including eEF1Bγ) [15].

The novel protein interaction network was generated by MCODE after complementing the BioGrid database with newly identified 234 nuclear partners of eEF1Bγ in A549 cells (Fig. 1). The resulting network contained 47 proteins (including eEF1Bγ). Several functional clusters comprising both experimentally defined and predicted by MCODE partners were observed. Cluster A comprised different polypeptides of polymerase (RNA)II (DNA directed) (POLR2B, POLR2C, POLR2D, POLR2G, POLR2E, POLR2J) involved in the transcription process. Importantly, direct interaction of eEF1Bγ with POLR2C was shown earlier [16] which presents independent evidence for eEF1Bγ contacts with the polymerase (RNA)II (DNA directed) complex. Cluster B included proteins ABCF1, EIF2B3, NMT1, EIF2B2, MRE11A, RFC4 and NELFB that are mainly involved in translation, transcription and DNA replication/reparation processes, Cluster C (TNFRSF10B, TNFRSF1A, FAS, FASLG, FADD, CASP10, CASP8, BID, MAPK8, RHOA, ARHDGIA, MSN, EZR) represented the membrane-related proteins involved in apoptosis, cell regulation and cytoskeleton-membrane interaction, Cluster D (PPP2CB, CTTNB2NL, STRN4, PDCD10) comprised the proteins involved in
Analysis of eEF1Bγ interactome in the nuclear fraction of A549 human lung adenocarcinoma cells

cell regulation, and Cluster E (eEF1Bβ, eIF4A3, eIF3M, PABPC1, SYNCRIp, HNRNPD, HNRNPH1, SNRNp20, MED23, SF3B1, SNRPA1, HNRNPH2, SRSF1, TADA2A, tat, gag-pol) included the proteins involved in translation, mRNA splicing, metabolism and transport, transcription activation as well as two proteins related to HIV-1 infection.

A complementary approach to identify the cellular processes potentially involving eEF1Bγ is to combine our eEF1Bγ co-immunoprecipitation data with the proteomic data derived from precise co-fractionation of the cellular proteins with eEF1Bγ, with subsequent analysis of the functions of the proteins common for both data sets. Precise co-fractionation is considered an alternative way to estimate a possibility of protein-protein interaction in cell [17]. Recently we used this approach to identify the proteins co-fractionated with the
eEF1Bβ subunit of eEF1B in human cancer cells [10]. For this aim the Mapofthecell database of the subcellular localization of the proteins in cancer human cells was employed [17].

Here we utilized the same approach to find out the proteins of the nuclear fraction of human cancer cells, co-fractionated with the eEF1Bγ subunit. To do this we used the whole list of 234 protein partners of eEF1Bγ identified by Co-IP and MS in the nuclear fraction to check a possibility of their co-fractionation with eEF1Bγ according to the Mapofthecell database. Indeed, nine protein partners were found to co-localize with eEF1Bγ during precise co-fractionation (Fig. 2). All these proteins showed mixed cyto-nuclear localization according to the Subcellular localization database COMPARTMENTS (https://compartments.jensenlab.org) which is consistent with their presence in the nuclear fraction of A549 cells. Interestingly, two groups of the eEF1Bγ partners separated by fractionation were observed on the map (Fig. 2). ELAVL1, TIA1, CD2AP, PCBP2 and DAZAP1 represented Group 1, while SERBP1, YTHDC2, YTHDF2 and GIGYF2 represented Group 2. Peculiarly, all but one proteins possessed RNA-binding properties.

Interestingly, these proteins showed quite different abundance in human cancer cells [17]. The copy number of the eEF1Bγ estimated as number of molecules per cell (11 587 782) was comparable with the amount of the PCBP2, SERBP1 and FLAVL1 proteins. The copy number of YTHDF2 and DAZAP1 was 15-fold, CD2AP and TIA1 about 40-fold, GIGYF2–64-fold and YTHDC2–141-fold lower that the amount of the eEF1Bγ molecules per cell.

Fig.2. The experimentally defined partners of nuclear eEF1Bγ (EEF1G) selected by “Mapofthecell” co-fractionation approach. Map 2 of “Mapofthecell” database was used.
The characteristics of the eEF1Bγ partners are given below.

Protein **PCBP2** (Poly(rC) binding protein 2) is a splicing factor [18], it participates in signal transduction pathways [19]. PCBP2 is a negative regulator of IRES-mediated mRNA translation [20]. PCBP2 is a well-known iron chaperon [21], it participates in regulation of antioxidant defence [22]. It is involved in apoptosis [23] and innate immune response systems [24,25].

PCBP2 is linked to the cancer development, in particular, it is overexpressed in pancreatic ductal adenocarcinoma [26], glioma [27], hepatocellular carcinoma [28]. It exemplifies pro-viral activity [29,30].

**CD2AP** (CD2 associated protein) is known actin/cytoskeletal regulator controlling actin organization and cellular migration [31]. It interacts with actin capping protein, directs it to different subcellular locations and modulates its activity via allosteric effects [32]. It regulates exosome cargo protein trafficking through the Golgi complex [33].

Mice with CD2AP deficiency showed such signs of glomerular disease as effacement and disorganization of the slit diaphragm, significant membrane dynamics and disrupted podocyte and endothelial integrity [34]. CD2AP participates in spermatogenesis [35]. This protein could be an important factor for Alzheimer’s disease development [36]. In particular, it controls Aβ generation in dendritic early endosomes [37]. Also, CD2AP exemplifies pro-viral activity, for instance it binds unstructured subunits of Chikungunya virus replicase [38] and stimulates chronic hepatitis C virus propagation and steatosis by disrupting insulin signaling [39].

**TIA1** (T cell intracellular antigen-1) is prion-related RNA-binding protein [40]. During arsenic stress inducing global shortening of 3'UTRs, TIA1 preferentially interacts with shorter 3’UTR sequences through U-rich motifs, correlating with stress granule association and mRNA decay of long 3’UTR isoforms [41]. TIA1 protein essentially contributes to the fidelity of mRNA maturation, translation, and RNA-stress-sensing pathways in human cells [42]. TIA1 is a key component of stress granules which is regulated by Zn2+ ions [43]. DNA damage due to mitogens activation promotes mRNA relocation and translation in part due to dissociation of Tia1 from its mRNA targets [44].

TIA1 promotes cancer progression in different tissues [45–48]. It involved in tau-mediated neurodegeneration [49,50]. TIA1 is linked to Amyotrophic Lateral Sclerosis [51] and Welander distal myopathy [52]. TIA1 exemplifies pro-viral activity [53].

Interestingly, both CD2AP and TIA1 are involved in HIPPO signaling system [54,55].

**ELAVL1** (ELAV-like protein 1) which is also called **HuR** (human antigen R), is an RNA-binding protein involved in differentiation and stress response that acts primarily by stabilizing mRNA targets [56–58]. It binds 3’-untranslated region of BECN1/Beclin1 mRNA regulating ferroptosis, recently recognized form of controlled cell death that is characterized by lipid peroxidation, in liver fibrosis [59]. Its binding to PARG mRNA positively affects DNA repair and increases resistance to PARP inhibitors [60]. HuR/ELAVL1 binding to SCN5A mRNA increases its stability, with subsequent reduction of arrhythmic risk in heart failure [61].
ELAVL1 is involved in telomerase function, as it associates with TERC and promotes the assembly of the TERC/TERT complex by facilitating TERC C106 methylation [62]. It participates in spermatogenesis [63].

There are a number of reports, which link pro-tumor activity of HuR/ELAVL1 with cancer of different localizations [64–68]. Surprisingly, HuR/ELAVL1 binding to different long non-coding RNAs induces opposite effect on the proliferation of different tumor cells [69,70].

HuR/ELAVL1 demonstrates pro-viral activity [71]. This protein is linked to Parkinson’s disease [72]. Disruption of ELAVL1/HuR nuclear export is consistent with the effects of inborn errors of vitamin B12 (cobalamin) metabolisms on brain development, neuroplasticity and myelin formation [73].

DAZAP1 (DAZ-associated protein 1) is an RNA-binding protein involved in mammalian development and spermatogenesis [74]. Knockdown or over-expression of DAZAP1 causes a cell proliferation defect while phosphorylation of its C-terminal domain which is sufficient to activate splicing is essential for the nuclear/cytoplasmic translocation of DAZAP1 [75]. DAZAP1 affects splicing of pre-mRNA [76]. It can regulate translation of mRNA as well [77].

SERBP1 (Serpine1 mRNA Binding Protein 1) binds different protein partners which participate in different cellular processes. For instance, it interacts with signaling protein RACK1 involved to signal transduction, mRNA splicing and translation and the cytoskeleton [78]. It interacts with SPIN1, a maternal protein containing Tudor-like domains, which is involved in regulating maternal transcripts to control meiotic resumption by controlling mRNA stability and/or translation [79]. One of the inhibitory mechanisms in this case is the occupation of the ribosomal mRNA entrance channel [80]. It binds dimers of activation-induced cytidine deaminase (AID) which may contribute to DNA-cleavage and recombination [81]. SERBP1 also affects DNA repair [82]. It is involved in transcriptional complex [83]. During stress SERBP1 is distributed simultaneously to cytoplasmic stress granules and nucleoli, two ribonucleoprotein-enriched subcellular compartments [84]. Cytoplasmic distribution can be regulated by methylation of its arginine residues [85].

SERBP1 was markedly upregulated in prostate cancer tissues and was significantly associated with tissue metastasis and Gleason score. The loss of miR-26a-5p promotes proliferation, migration, and invasion through targeting SERBP1 [86].

GIGYF2 (GRB10 Interacting GYF Protein 2) is a specific RNA-binding protein linked to repression of translation. It shows at least two distinct mechanisms of repression: one depends on 4EHP binding and mainly affects translation; the other is 4EHP-independent and involves the CCR4/NOT complex and its deadenylation activity [87]. Protein GIGYF2 is also a regulator of miRNA-mediated translation repression [88]. GIGYF2 is an autophagy regulator controlling neuron and muscle homeostasis [89] which is possibly involved in the regulation of signaling at endosomes [90]. It is also involved in mammalian development [91].

GIGYF2 mutations may be associated with increased risk of Parkinson’s disease [92, 93] and macrocephaly [94]. Increased expression
of GIGYF2 might contribute to the development of diabetes-associated cognitive disorder via negatively regulating IGF1R signaling pathway [95].

**YTHDC2** (YTH Domain Containing 2) protein is a N6-methyladenosine (m6A) reader that specifically recognizes and binds modified nucleotides in RNA. YTHDC2 enhances the translation efficiency of its targets and also decreases their mRNA abundance [96]. Recently it was shown that YTHDC2 interacts with the small ribosomal subunit in close proximity to the mRNA entry/exit sites and controls specific mRNAs by recruitment of the RNA degradation machinery to regulate the stability of m6A-containing mRNAs and by utilizing its distinct RNA-binding domains to bridge interactions between m6A-containing mRNAs and the ribosomes to facilitate their efficient translation [97]. Regulation of gene expression by YTHDC2 is considered an evolutionarily ancient strategy for controlling the germline transition into meiosis [98, 99]. YTHDC2 is found to regulate mammalian spermatogenesis [96]. YTHDC2 promotes cancer metastasis [100].

**YTHDF2** (YTH N6-Methyladenosine RNA Binding Protein 2) is another m6A reader which reduces the stability of target transcripts [101]. Due to this, YTHDF2 plays a role in maternal-to-zygotic transition during the early life of embryos [102, 103]. YTHDF2 may recognize and bind the m6A site of FAM134B that plays a pivotal role in lipid homeostasis to reduce its mRNA lifetime and reduce its protein abundance [104]. It binds to the peroxisome proliferator-activated receptor α to mediate its mRNA stability to regulate lipid metabolism [105]. It recognized and decayed methylated mRNAs of Cyclin-A2 and kinase CDK2, thereby prolonging cell cycle progression and suppressing adipogenesis [106]. YTHDF2 plays an important role in regulating hematopoietic stem cells ex vivo expansion by regulating the stability of multiple mRNAs critical for HSC self-renewal of these cells [107]. Ythdf2 modulates neural development by promoting m6A-dependent degradation of neural development-related mRNA target [108]. YTHDF2 is mainly present in the cytosol, however, nearly all YTHDF2 translocated from the cytosol into the nucleus after heat shock [109].

YTHDF2 is a negative regulator of interferon response as it facilitates the fast turnover of interferon mRNAs and consequently helps viral propagation [110]. YTHDF2 plays positive roles in viral gene expression and HIV-1 particle assembly, suggesting that HIV-1 interacts with mRNA decay components to successfully accomplish viral replication [111, 112]. Overexpression of YTHDF2 induces more rapid viral replication, and larger viral plaques, in SV40 infected BSC40 cells [113]. YTHDF2 directly binds the m6A modification site of EGFR 3'-UTR to promote the degradation of EGFR mRNA in HCC cells acting as a tumor suppressor to repress cancer cell proliferation and growth [114,115].

The information is summarized in Table 1. Supplementation of the bioinformatic Cytoscape approach with the experimental pull-down data permitted to pinpoint a number of different cellular processes potentially involving eEF1Bγ in nucleus. Among those could be the main molecular biological processes: replication/reparation, transcription and translation. According to Cytoscape, eEF1Bγ
may take part in apoptosis, cell regulation and cytoskeleton-membrane interaction, including exosomal trafficking. Use of the Mapofthecell database relying on precise cellular sub-fractionation, provided a basis for narrowing the wide functional variety of potential functions of eEF1Bγ expected from a number of its cellular partners in nuclear fraction. Consequently, we analyzed the functions of the experimentally proved eEF1Bγ partners which were predicted by both Cytoscape and Mapofthecell approaches. We reasoned that the coincidence of the functions of the eEF1Bγ partners identified by different approaches increases the probability of eEF1Bγ involvement in fulfillment of this function.

Peculiarly, two functions based on the mRNA-binding properties of the proteins were markedly represented in both datasets. The first one is the splicing of mRNA presented by HNRNPH1, HNRNPD, SNRNP20, MED23, SF3B1 (Cytoscape) and PCBP2, DAZAP1 (MapoftheCell). Fig. 3 shows a possibility that HNRNPD, DAZAP1 and eEF1Bγ on one side and HNRNPH1, SNRNP20, MED23, SF3B1 and PCBP2 on another side can be involved in different entities in the cell so, the interaction of eEF1Bγ with the second formation can

Table 1. The Co-IP/MS identified protein partners, which were co-fractionated with eEF1Bγ.

| №  | Gene names | Protein names                                           | Copy number/cell | RNA-binding ability | Localization | Relation to diseases                                      |
|----|------------|---------------------------------------------------------|------------------|---------------------|--------------|----------------------------------------------------------|
| 1  | PCBP2      | Poly(RC) Binding Protein 2                              | 13,998,300       | +                   | ++++         | Pro-tumor activity, pro-viral activity                   |
| 2  | CD2AP      | CD2 Associated Protein                                   | 276,039          | ++++                | ++++         | glomerular disease, Alzheimer’s disease, pro-viral activity |
| 3  | TIA1       | Cytotoxic Granule Associated RNA Binding Protein         | 282,879          | +                   | ++++         | Pro-tumor activity, Alzheimer’s disease, Amyotrophic Lateral Sclerosis, Welander Distal Myopathy |
| 4  | ELAVL1 (HUR) 53 | ELAV Like RNA Binding Protein 1                      | 3,678,805        | +                   | ++++         | Pro-tumor activity, pro-viral activity, Parkinson’s Disease, Inherited disorders of cobalamin metabolism |
| 5  | DAZAP1 48  | DAZ Associated Protein 1                                 | 778,860          | +                   | ++++         | azoospermia                                              |
| 6  | SERBP1     | SERPINE1 mRNA Binding Protein 1                          | 9,859,640        | +                   | ++++         | Pro-tumor activity                                      |
| 7  | YTHDC2     | YTH Domain Containing 2                                  | 82,311           | +                   | +++         | Pro-tumor activity                                      |
| 8  | GiGYF2     | GRB10 Interacting GYF Protein 2                          | 180,458          | +                   | +++         | Macrocephaly, Parkinson disease                         |
| 9  | YTHDF2     | N6-Methyladenosine RNA Binding Protein 2                 | 723,343          | +                   | ++++        | tumor suppressor, pro-viral activity                    |
be dynamic rather than stable. Thus, nucleus-localized eEF1Bγ can be involved in splicing events. Importantly, the experimental proteomic data have suggested that eEF1Bγ could be a member of pre-mRNA 3’ processing complex [116].

The second function is the regulation of stability of mRNA which involves HNRNPD, HNRNPH1 (Cytoscape) and GIGYF2, YTHDC2, YTHDF2, TIA1, ELAVL1 (MapoftheCell). MapoftheCell of these partners shows that HNRNPH1 does not seem to belong to the stable complex (Fig. 4) which cannot exclude, however, the existence of the dynamic interaction between HNRNPH1 and other members of the entity. One may suggest that eEF1Bγ can be bound to different mRNAs in nucleus contributing to their stability and transport. Notably, eEF1Bγ has been described to bind the 3’ UTRs of vimentin [117] and some other mRNA [16].

Another function of eEF1Bγ partners identified by both approaches is related to cytoskeleton-membrane link and cellular trafficking. The group comprises EZR and HNRNPH1 (Cytoscape) and CD2AP (MapoftheCell). It is widely accepted that ezrin participates in anchoring membrane proteins to the cortical actin network [118]. Nuclear localization of ezrin was also reported, however, the role of nuclear ezrin is not yet deciphered [119]. HNRNPH1 and CD2AP participate in exosome trafficking [33,120]. It is worthy to mention that a role of eEF1Bγ in organelle transport has been shown [16,121]. eEF1Bγ was also co-immunoprecipitated with an essential component of ER-Golgi transport vesicles [122]. Also, it is known that eEF1Bγ interacts with cytoskeleton [123] and membranes [124]. All these facts permit to suggest that eEF1Bγ, along with its partners, may contribute to the cytoskeleton-membrane interaction and perform a transport role.

Importantly, the majority of described protein partners of eEF1Bγ in the nuclear fraction are strongly linked to cancer. Cancer-related functions of PCBP2, TIA-1, HuR/ELAVL1, SERBP1, YTHDC2, YTHDF2 (MapoftheCell database) were described above. All experimentally defined partners of eEF1Bγ picked up by Cytoscape to build molecular networks, are linked to cancer as well [2,125–132]. Based on these data one may suggest that eEF1Bγ is also associated with cancer and may play a central hub role to link together various cancer-related processes. There are a few experimental facts indicating such a possibility [7,8,133]. One may suggest that translation function of eEF1Bγ is linked to its involvement to eEF1B complex, while the induced by cancer appearance of free eEF1Bγ has regulatory consequences relying on its ability to influence pre-mRNA splicing and mRNAs stability. Subsequently, eEF1Bγ could be a novel perspective target for molecular therapy of cancer.

Another function of eEF1Bγ and its partners can be associated with viral propagation. Interestingly, 55 % of the eEF1Bγ partners picked up by MapoftheCell and 37 % of the eEF1Bγ partners picked up by Cytoscape showed pro- or anti-viral activity. Peculiarly, eEF1Bγ by itself is involved in viral infection and propagation [134–136].

Interestingly, no indication of the nuclear eEF1Bγ partners link to retinoblastoma was found, contrary to what was observed for cytoplasmic eEF1Bγ [15]. In contrast, a number
Fig. 3. Protein partners of nuclear eEF1Bγ involved in splicing of pre-mRNA. Experimentally defined partners of eEF1Bγ (depicted in blue) predicted by Cytoscape (in red) and Mapofthecell (in green). Numbers show different groups of the partners described in the text. Map 3 of “Mapofthecell” database was used.

Fig. 4. Protein partners of nuclear eEF1Bγ involved in regulation of mRNA stability. Experimentally defined partners of eEF1Bγ (depicted in blue) predicted by Cytoscape (in red) and Mapofthecell (in green). Map 3 of “Mapofthecell” database was used.
of eEF1Bγ partners in the nuclear fraction were shown to be related to neurodegenerative disorders (Alzheimer’s disease, Parkinson’s disease, epilepsy, intellectual disability) so, possible involvement of nuclear eEF1Bγ in genesis of these diseases should be elucidated in future studies.

**Conclusions**

234 proteins were identified by co-immunoprecipitation and LC-MS-MS as interacting with eEF1Bγ in the nuclear fraction of lung cancer cells A549. Possible functional networks involving eEF1Bγ and its partners were built with the use of the Cytoscape 3.2.0 program. The networks were related to the DNA replication/reparation, transcription, translation, cell regulation and cytoskeleton-membrane interaction, mRNA splicing and intracellular transportation processes. Additional analysis with Mapofthecell engine based on precise protein co-fractionation, permitted to pinpoint two main processes in which nuclear eEF1Bγ may be involved. They are splicing of mRNA and regulation of mRNA stability. According to our data, eEF1Bγ may also take part in cytoskeleton-membrane linking and cellular trafficking.

**Acknowledgments**

This work was partially financed by the Interdisciplinary Program of Scientific research of NAS of Ukraine “Molecular and cell biotechnologies for medicine, industry and agriculture” and Collaborative Program of NAS of Ukraine and PAN 2018-2020. This work was supported by the state program “Support of the development of priority directions of scientific research” (KPIKBK 6541230).

We thank Prof. Anna El’skaya for her valuable contribution to the preparation of the manuscript. We thank Prof. M. Dadlez for an expert help in performing MS analysis. The contribution of V. Zakon and Dr. I. Groisman to the MS data analysis is appreciated. We are grateful to M. M. Ilchenko for helpful advice.

**REFERENCES**

1. Trosiuk TV, Shalak VF, Szczepanowski RH, Negrutskii BS, El’skaya AV. A non-catalytic N-terminal domain negatively influences the nucleotide exchange activity of translation elongation factor 1Balpa. FEBS J. 2015; 283(3): 484–97.
2. Novosylna OV, Timchenko AA, Tiktopulo EI, Serdyuk IN, Negrutskii BS, El’skaya AV. Characterization of physical properties of two isoforms of translation elongation factor 1A. Biopolym Cell. 2007; 23(5): 386–90.
3. 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(32): 5345–53.
4. Negrutskii B, Vlasenko D, Mirande M, Futernyk P, El’skaya A. mRNA-Independent way to regulate translation elongation rate in eukaryotic cells. IUBMB Life. 2018; 70(3): 192–6.
5. Janssen GM, Moller W. Kinetic studies on the role of elongation factors 1 beta and 1 gamma in protein synthesis. J Biol Chem. 1988; 263(4): 1773–8.
6. Janssen GM, van Damme HT, Kriek J, Amons R, Moller W. The subunit structure of elongation factor 1 from Artemia Why two alpha-chains in this complex? J Biol Chem 1994; 269(50): 31410–7.
7. Veremieva M, Khoruzhenko A, Zaicev S, Negrutskii B, El’skaya A. Unbalanced expression of the translation complex eEF1 subunits in human cardioesophageal carcinoma. Eur J Clin Invest. 2011; 41(3): 269–76.
8. Veremieva M, Kapustian L, Khoruzhenko A, Zakharychev V, Negrutskii B, El’skaya A. Independent overexpression of the subunits of translation
elongation factor complex eEF1H in human lung cancer. BMC Cancer. 2014; 14913.
9. Nathanson L, Xia T, Deutscher MP. Nuclear protein synthesis: a re-evaluation. RNA. 2003; 9(1): 9–13.
10. Kapustian LM, Dadlez M, Negrutskii BS. Protein partners of the eEF1Bβ subunit of the translation elongation complex eEF1B in the nuclear fraction of human lung carcinoma cells. Biopolym Cell. 2017; 33(4): 243–55.
11. Kang D, Gho Y, Suh M, Kang C. Highly Sensitive and Fast Protein Detection with Coomassie Brilliant Blue in Sodium Dodecyl Sulfate-Polyacrylamide Gel Electrophoresis. Commun to Ed Bull Korean Chem Soc. 2002; 23(11): 1511–12.
12. Kapustian LM, Dadlez M, Negrutskii BS. Non-canonical interactions of the β subunit of the translation elongation complex eEF1B and analysis of their possible functional role. Biopolym Cell. 2016; 32(5): 347–58.
13. Shannon P, Markiel A, Ozier O, Baliga NS, Wang JT, Ramage D, Amin N, Schwikowski B, Ideker T. Cytoscape: a software environment for integrated models of biomolecular interaction networks. Genome Res. 2003; 13(11): 2498–504.
14. Bader GD, Hogue CWV. An automated method for finding molecular complexes in large protein interaction networks. BMC Bioinformatics. 2003; 42.
15. Kapustian LM, Lysetsky IL, Bondarchuk TV. Mass-spectrometric and bioinformatic analysis of eEF1By interactome in the cytoplasmic fraction of A549 cells. Biopolym Cell. 2018; 34(4): 292–302.
16. Pisani C, Onori A, Gabanella F, Delle Monache F, Borreca A, Ammassari-Teule M, Fanciulli M, Di Certo MG, Passananti C, Corbi N. eEF1Bgamma binds the Che-1 and TP53 gene promoters and their transcripts. J Exp Clin Cancer Res. 2016; 35(1): 146.
17. Itzhak DN, Tyanova S, Cox J, Borner GH. Global, quantitative and dynamic mapping of protein subcellular localization. Elife. 2016; 5.
18. Ghanem LR, Kromer A, Silverman IM, Ji X, Gazzara M, Nguyen N, Aguilar G, Martinelli M, Barash Y, Liebhaber SA. Poly(C)-Binding Protein Pebp2 Enables Differentiation of Definitive Erythropoiesis by Directing Functional Splicing of the Runx1 Transcript. Mol Cell Biol. 2018; 38(16): e00175-18.
19. Chen C, Lei J, Zheng Q, Tan S, Ding K, Yu C. Poly(rC) binding protein 2 (PCBP2) promotes the viability of human gastric cancer cells by regulating CDK2. FEBS Open Bio. 2018; 8(5): 764–73.
20. Kim J-K, Kim I, Choi K, Choi J-H, Kim E, Lee H-Y, Park J, Kim Yoon S. Poly(rC) binding protein 2 acts as a negative regulator of IRES-mediated translation of Hr mRNA. Exp Mol Med. 2018; 50(2): e441.
21. Yanatori I, Richardson DR, Toyokuni S, Kishi F. The iron chaperone poly(rC)-binding protein 2 forms a metabolon with the heme oxygenase 1/cytochrome P450 reductase complex for heme catabolism and iron transfer. J Biol Chem. 2017; 292(32): 13205–29.
22. Ren C, Zhang J, Yan W, Zhang Y, Chen X. RNA-binding Protein PCBP2 Regulates p73 Expression and p73-dependent Antioxidant Defense. J Biol Chem. 2016; 291(18): 9629–37.
23. Mao X, Liu J, Chen C, Zhang W, Qian R, Chen X, Lu H, Ge J, Zhao C, Zhang D, Wang Y. PCBP2 Modulates Neural Apoptosis and Astrocyte Proliferation After Spinal Cord Injury. Neurochem Res. 2016; 41(9): 2401–14.
24. Qin Y, Xue B, Liu C, Wang X, Tian R, Xie Q, Guo M, Li G, Yang D, Zhu H. NLRX1 mediates MAVS degradation to attenuate hepatitis C virus-induced innate immune response through PCBP2. J Virol. 2017; 91(23): e01264-17.
25. Lee E-Y, Lee H-C, Kim H-K, Jang SY, Park S-J, Kim Y-H, Kim JH, Hwang J, Kim J-H, Kim T-H, Arif A, Kim S-Y, Choi Y-K, Lee C, Lee C-H, Jung JU, Fox PL, Kim S, Lee J-S, Kim MH. Infection-specific phosphorylation of glutamyl-prolyl tRNA synthetase induces antiviral immunity. Nat Immunol. 2016; 17(11): 1252–62.
26. Wan C, Gong C, Zhang H, Hua L, Li X, Chen X, Chen Y, Ding X, He S, Cao W, Wang Y, Fan S, Xiao Y, Zhou G, Shen A. beta2-adrenergic receptor signaling promotes pancreatic ductal adenocarcinoma (PDAC) progression through facilitating PCBP2-dependent c-myc expression. Cancer Lett. 2016; 373(1): 67–76.
27. Tang S-L, Gao Y-L, Chen X-B. MicroRNA-214 targets PCBP2 to suppress the proliferation and growth of glioma cells. Int J Clin Exp Pathol. 2015; 8(10): 12571–6.
28. Zhang X, Hua L, Yan D, Zhao F, Liu J, Zhou H, Liu J, Wu M, Zhang C, Chen Y, Chen B, Hu B. Overexpression of PCBP2 contributes to poor prognosis and enhanced cell growth in human hepatocellular carcinoma. Oncol Rep. 2016; 36(6): 3456–64.

29. Asnani M, Pestova TV, Hellen CUT. PCBP2 enables the cadicivirus IRES to exploit the function of a conserved GRNA tetraloop to enhance ribosomal initiation complex formation. Nucleic Acids Res. 2016; 44(20): 9902–17.

30. Lopez-Manriquez E, Vashist S, Urena L, Goodfellow I, Chavez P, Mora-Heredia JE, Cancio-Lonches C, Garrido E, Gutierrez-Escolano AL. Norovirus genome circularization and efficient replication are facilitated by binding of PCBP2 and hnRNP A1. J Virol. 2013; 87(11): 11371–87.

31. Cummins TD, Wu KZL, Bozatzi P, Dingwell KS, Macartney TJ, Wood NT, Varghese J, Gourlay R, Campbell DG, Prescott A, Griffis E, Smith JC, Sapkota GP: PAWS1 controls cytoskeletal dynamics and cell migration through association with the SH3 adaptor CD2AP. J Cell Sci. 2018; 131(1): jcs202390.

32. Edwards M, Zwolak A, Schafer DA, Sept D, Dominguez R, Cooper JA. Capping protein regulators fine-tune actin assembly dynamics. Nat Rev Mol Cell Biol. 2014; 15(10): 677–89.

33. Kwon S-H, Oh S, Nacke M, Mostov KE, Lipp schutz JH. Adaptor protein CD2AP and L-type lectin LMAN2 regulate exosome cargo protein trafficking through the Golgi complex. J Biol Chem. 2017; 292(40): 16523.

34. Tsuji K, Paunescu TG, Suleiman H, Xie D, Mamuya FA, Miner JH, Lu HAJ. Re-characterization of the Glomerulopathy in CD2AP Deficient Mice by High-Resolution Helium Ion Scanning Microscopy. Sci Rep. 2017; 7(1): 8321.

35. Xia W, Mruk DD, Lee WM, Cheng CY. Differential interactions between transforming growth factor-beta3/TbetaR1, TAB1, and CD2AP disrupt blood-testis barrier and Sertoli-germ cell adhesion. J Biol Chem. 2006; 281(24): 16799–813.

36. Dubey H, Guilati K, Ray A. Recent studies on cellular and molecular mechanisms in Alzheimer’s disease: focus on epigenetic factors and histone deacetylase. Rev Neurosci. 2018; 29(3): 241–60.

37. Ubelmann F, Burrinha T, Salavessa L, Gomes R, Ferreira C, Moreno N, Guimas Almeida C. Bin1 and CD2AP polarise the endocytic generation of beta-amyloid. EMBO Rep. 2017; 18(1): 102–22.

38. Mutso M, Morro AM, Smedberg C, Kasvandik S, Aquilimeba M, Teppor M, Tarve L, Lulla A, Lulla V, Saul S, Thaa B, McNerney GM, Merits A, Varjak M. Mutation of CD2AP and SH3KBP1 Binding Motif in Alphavirus nsP3 Hypervariable Domain Results in Attenuated Virus. Viruses. 2018; 10(5): 226.

39. Zhang H, Zhang C, Tang H, Gao S, Sun F, Yang Y, Zhou W, Hu Y, Ke C, Wu Y, Ding Z, Guo L, Pei R, Chen X, Sy M-S, Zhang B, Li C. CD2-Associated Protein Contributes to Hepatitis C, Virus Propagation and Steatosis by Disrupting Insulin Signaling. Hepatology. 2018; 68(5): 1710–25.

40. Rayman JB, Kandel ER. TIA-1 Is a Functional Prion-Like Protein. Cold Spring Harb Perspect Biol. 2017; 9(5): a030718.

41. Zheng D, Wang R, Ding Q, Wang T, Xie B, Wei L, Zhong Z, Tian B. Cellular stress alters 3'UTR landscape through alternative polyadenylation and isoform-specific degradation. Nat Commun. 2018; 9(1): 2268.

42. Meyer C, Garzia A, Mazzola M, Gerstberger S, Molina H, Tuschi T. The TIA1 RNA-Binding Protein Family Regulates EIF2AK2-Mediated Stress Response and Cell Cycle Progression. Mol Cell. 2018; 69(4): 622–635.e6.

43. Rayman JB, Karl KA, Kandel ER. TIA-1 Self-Multimerization, Phase Separation, and Recruitment into Stress Granules Are Dynamically Regulated by Zn(2). Cell Rep. 2018; 22(1): 59–71.

44. Diaz-Munoz MD, Kiselev VY, Le Novere N, Curk T, Ule J, Turner M. Tial dependent regulation of mRNA subcellular location and translation controls p53 expression in B cells. Nat Commun. 2017; 8(1): 530.

45. Yang X, Wang M, Lin B, Yao D, Li J, Tang X, Li S, Liu Y, Xie R, Yu S. miR-487a promotes progression of gastric cancer by targeting TIA1. Biochimie. 2018; 154119–26.

46. Tak H, Kang H, Ji E, Hong Y, Kim W, Lee EK. Potential use of TIA-1, MFF, microRNA-200a-3p, and microRNA-27 as a novel marker for hepatocellular carcinoma. Biochem Biophys Res Commun. 2018; 497(4): 1117–22.
47. Liu Y, Liu R, Yang F, Cheng R, Chen X, Cui S, Gu Y, Sun W, You C, Liu Z, Sun F, Wang Y, Fu Z, Ye C, Zhang C, Li J, Chen X. miR-19a promotes colorectal cancer proliferation and migration by targeting TIA1. Mol Cancer. 2017; 16(1): 53.

48. Hamada J, Shoda K, Masuda K, Fujita Y, Naruto T, Kohmoto T, Miyakami Y, Watanabe M, Kudo Y, Fujiiwa H, Ichikawa D, Otsuji E, Imoto I. Tumor-promoting function and prognostic significance of the RNA-binding protein T-cell intracellular antigen-1 in esophageal squamous cell carcinoma. Oncotarget. 2016; 7(13): 17111–28.

49. Jiang L, Ash PEA, Maziuk BF, Ballance HI, Boudeau S, Abdullatif A Al, Orlando M, Petrucelli L, Ikezu T, Wolozin B. TIA1 regulates the generation and response to toxic tau oligomers. Acta Neuropathol. 2019; 137(2): 259–77.

50. Apicco DJ, Ash PEA, Maziuk B, LeBlang C, Medalla M, Al Abdullatif A, Ferragud A, Botelho E, Ballance HI, Dhawan U, Boudeau S, Cruz AL, Kashy D, Wong A, Goldberg LR, Yazdani N, Li H, Luebke J, Bryant CD, Wolozin B. Reducing the RNA binding protein TIA1 protects against tau-mediated neurodegeneration in vivo. Nat Neurosci. 2018; 21(1): 72–80.

51. Zhao M, Kim JR, van Bruggen R, Park J. RNA-Binding Proteins in Amyotrophic Lateral Sclerosis. Mol Cells. 2018; 41(9): 818–29.

52. Brand P, Dyck PJB, Liu J, Berini S, Selcen D, Milone M. Distal myopathy with coexisting heterozygous TIA1 and MYH7 Variants. Neuromuscul Disord. 2016; 26(8): 511–5.

53. Sun Y, Dong L, Yu S, Wang X, Zheng H, Zhang P, Meng C, Zhan Y, Tan L, Song C, Qiu X, Wang G, Liao Y, Ding C. Newcastle disease virus induces stable formation of bona fide stress granules to facilitate viral replication through manipulating host protein translation. FASEB J Off Publ Fed Am Soc Exp Biol. 2017; 31(4): 1337–53.

54. Ma X, Wang H, Ji J, Xu W, Sun Y, Li W, Zhang X, Chen J, Xue L. Hippo signaling promotes JNK-dependent cell migration. Proc Natl Acad Sci U S A. 2017; 114(8): 1934–9.

55. Li F, Bullough KZ, Vashisht AA, Wohlschlegel JA, Philpott CC. Poly(rC)-Binding Protein 2 Regulates Hippo Signaling To Control Growth in Breast Epithelial Cells. Mol Cell Biol. 2016; 36(16): 2121–31.

56. Pabis M, Popowicz GM, Stehle R, Fernandez-Ramos D, Asami S, Warner L, Garcia-Maurino SM, Schlundt A, Martinez-Chantar ML, Díaz-Moreno I, Sattler M. HuR biological function involves RRM3-mediated dimerization and RNA binding by all three RRM. Nucleic Acids Res. 2019; 47(2): 1011–29.

57. Zhou A, Shi G, Kang G-J, Xie A, Liu H, Jiang N, Liu M, Jeong E-M, Dudley SCJ. RNA Binding Protein, HuR, Regulates SCN5A Expression Through Stabilizing MEF2C transcription factor mRNA. J Am Heart Assoc. 2018; 7(9): e007802.

58. Zybura-Broda K, Wolder-Gontarek M, Ambrozek-Latecka M, Choros A, Bogusz A, Wilemska-Dziaduszyczka J, Rylski M. HuR (Elavl1) and HuB (Elavl2) Stabilize Matrix Metalloproteinase-9 mRNA During Seizure-Induced Mmp-9 Expression in Neurons. Front Neurosci. 2018; 12224.

59. Zhang Z, Yao Z, Wang L, Ding H, Shao J, Chen A, Zhang F, Zheng S. Activation of ferritinophagy is required for the RNA-binding protein ELAVL1/ HuR to regulate ferroptosis in hepatic stellate cells. Autophagy. 2018; 14(12): 2083–103.

60. Chand SN, Zarei M, Schiewer MJ, Kamath AR, Romeo C, Lal S, Cozziorto JA, Nevler A, Scolaro L, Londin E, Jiang W, Meisner-Kober N, Pishvaian MJ, Knudsen KE, Yeo CJ, Pascal JM, Winter JM, Brody JR. Posttranscriptional Regulation of PARG mRNA by HuR Facilitates DNA Repair and Resistance to PARP Inhibitors. Cancer Res. 2017; 77(18): 5011–25.

61. Zhou A, Xie A, Kim TY, Liu H, Shi G, Kang G-J, Jiang N, Liu M, Jeong E-M, Choi B-R, Dudley SCJ. HuR-mediated SCN5A messenger RNA stability reduces arrhythmic risk in heart failure. Hear Rhythm. 2018; 15(7): 1072–80.

62. Tang H, Wang H, Cheng X, Fan X, Yang F, Zhang M, Chen Y, Tian Y, Liu C, Shao D, Jiang B, Dou Y, Cong Y, Xing J, Zhang X, Yi X, Songyang Z, Ma W, Zhao Y, Wang X, Ma J, Gorospe M, Ju Z, Wang W. HuR regulates telomerase activity through TERC methylation. Nat Commun. 2018; 9(1): 2213.
63. Chi MN, Auriol J, Jegou B, Kontoyiannis DL, Turner JMA, de Rooij DG, Morello D. The RNA-binding protein ELAVL1/HuR is essential for mouse spermatogenesis, acting both at meiotic and postmeiotic stages. *Mol Biol Cell.* 2011; 22(16): 2875–85.

64. Zarei M, Lal S, Vaziri-Gohar A, O’Hayer K, Gundu V, Singh PK, Brody JR, Winter JM. RNA-Binding Protein HuR Regulates Both Mutant and Wild-Type IDH1 in IDH1-Mutated Cancer. *Mol Cancer Res.* 2019; 17(2): 508–20.

65. Levidou G, Kotta-Loizou I, Tasoulas J, Papadopoulos T, Theocharis S. Clinical Significance and Biological Role of HuR in Head and Neck Carcinomas. *Dis Markers.* 2018; 20184020937.

66. Al-Haidari A, Algaber A, Madhi R, Syk I, Thorlacius H. MiR-155-5p controls colon cancer cell migration via post-transcriptional regulation of Human Antigen R (HuR). *Cancer Lett.* 2018; 421145–51.

67. Brody JR, Dixon DA. Complex HuR function in pancreatic cancer cells. *Wiley Interdiscip Rev RNA.* 2018; 9(3): e1469.

68. Liu G, Grant WM, Persky D, Latham VMJ, Singer RH, Condeelis J. Interactions of elongation factor 1alpha with F-actin and beta-actin mRNA: implications for anchoring mRNA in cell protrusions. *Mol Biol Cell.* 2002; 13(2): 579–92.

69. Lei W, Wang Z-L, Feng H-J, Lin X-D, Li C-Z, Fan D. Long non-coding RNA SNHG12 promotes the proliferation and migration of glioma cells by binding to HuR. *Int J Oncol.* 2018; 53(3): 1374–84.

70. Lan Y, Xiao X, He Z, Luo Y, Wu C, Li L, Song X. Specific intron-dependent loading of DAZAP1 onto the cox6c transcript suppresses pre-mRNA splicing efficiency and induces cell growth retardation. *Gene.* 2018; 6571–8.

71. Smith RWP, Anderson RC, Smith JWS, Brook M, Richardson WA, Gray NK. DAZAP1, an RNA-binding protein required for development and spermatogenesis, can regulate mRNA translation. *RNA.* 2011; 17(7): 1282–95.

72. Bolger GB. The RNA-binding protein SERBP1 interacts selectively with the signaling protein RACK1. *Cell Signal.* 2017; 35256–63.

73. Battaglia-Hsu S-F, Ghemrawi R, Coelho D, Dreu-mont N, Mosca P, Hergalan S, Gauchotte G, Sequeira JM, Ndiongou M, Houlgatte R, Alberto J-M, Umoret R, Robert A, Paoli J, Jung M, Quadros E Y, Gueant J-L. Inherited disorders of cobalamin metabolism disrupt nucleocytoplasmic transport of mRNA through impaired methylation/phosphorylation of ELAVL1/HuR. *Nucleic Acids Res.* 2018; 46(15): 7844–57.

74. Yang C-K, Chen P. Differential translation of Dazap1 transcripts during spermatogenesis. *PLoS One.* 2013; 8(4): e60873.

75. Choudhury R, Roy SG, Tsai YS, Tripathy A, Graves LM, Wang Z. The splicing activator DAZAP1 integrates splicing control into MEK/Erk-regulated cell proliferation and migration. *Nat Commun.* 2014; 53078.

76. Sasaki K, Ono M, Takabe K, Suzuki A, Kurihara Y. Specific intron-dependent loading of DAZAP1 onto the cox6c transcript suppresses pre-mRNA splicing efficiency and induces cell growth retardation. *Gene.* 2018; 6571–8.

77. Smith RWP, Anderson RC, Smith JWS, Brook M, Richardson WA, Gray NK. DAZAP1, an RNA-binding protein required for development and spermatogenesis, can regulate mRNA translation. *RNA.* 2011; 17(7): 1282–95.

78. Bolger GB. The RNA-binding protein SERBP1 interacts selectively with the signaling protein RACK1. *Cell Signal.* 2017; 35256–63.

79. Chew TG, Peaston A, Lim AK, Lorthongpanich C, Knowles BB, Solter D. A tudor domain protein SPINDLIN1 interacts with the mRNA-binding protein SERBP1 and is involved in mouse oocyte meiotic resumption. *PLoS One.* 2013; 8(7): e69764.

80. Brown A, Baird MR, Yip MC, Murray J, Shao S. Structures of translationally inactive mammalian ribosomes. *Elife.* 2018; 7: e40486.

81. Mondal S, Begum NA, Hu W, Honjo T. Functional requirements of AID’s higher order structures and their interaction with RNA-binding proteins. *Proc Natl Acad Sci U S A.* 2016; 113(11): E1545–54.

82. Ahr J-W, Kim S, Na W, Baek S-J, Kim J-H, Min K, Yeom J, Kwak H, Jeong S, Lee C, Kim S-Y, Choi CY. SERBP1 affects homologous recombination-mediated
DNA repair by regulation of CtIP translation during S phase. Nucleic Acids Res. 2015; 43(13): 6321–33.

83. Mari Y, West GM, Scharager-Tapia C, Pascal BD, Garcia-Ordonez RD, Griffin PR. SERBP1 is a Component of the Liver Receptor Homologue-1 Transcriptional Complex. J Proteome Res. 2015; 14(11): 4571–80.

84. Lee Y-J, Wei H-M, Chen L-Y, Li C. Localization of SERBP1 in stress granules and nucleoli. FEBS J. 2014; 281(1): 352–64.

85. Lee Y-J, Hsieh W-Y, Chen L-Y, Li C. Protein arginine methylation of SERBP1 by protein arginine methyltransferase 1 affects cytoplasmic/nuclear distribution. J Cell Biochem. 2012; 113(8): 2721–8.

86. Guo K, Zheng S, Xu Y, Xu A, Chen B, Xun G, Ou J, Chen B, Duan G, Bai T, Zhao N, Shen Y, Li Y, Wang Y, Zhang Y, Baker C, Liu Y, Pang N, Huang L, Han L, Jia X, Liu C, Ni H, Yang X, Xia L, Chen J, Shen L, Li Y, Zhao R, Zhao W, Peng J, Pan Q, Long Z, Su W, Tan J, Du X, Ke X, Yao M, Hu Z, Zou X, Zhao J, Bernier RA, Eichler EE, Xia K. Inherited and multiple de novo mutations in autism/developmental delay risk genes suggest a multifactorial model. Mol Autism. 2018; 964.

88. Schopp IM, Amaya Ramirez CC, Debeljak J, Kreibich E, Skribbe M, Wild K, Bethune J. Split-BioID a conditional proteomics approach to monitor the composition of spatiotemporally defined protein complexes. Nat Commun. 2017; 8: 815690.

89. Kim M, Semple I, Kim B, Kiers A, Nam S, Park H-W, Park H, Ro S-H, Kim J-S, Juhasz G, Lee JH. Drosophila Gyl/GRB10 interacting GYF protein is an autophagy regulator that controls neuron and muscle homeostasis. Autophagy. 2015; 11(8): 1358–72.

100. Tanabe A, Tanikawa K, Tsunetomi M, Takai K, Ikeda H, Konno J, Tortigoe T, Maeda H, Kutomi G,
Okita K, Mori M, Sahara H. RNA helicase YTHDC2 promotes cancer metastasis via the enhancement of the efficiency by which HIF-1alpha mRNA is translated. Cancer Lett. 2016; 376(1): 34–42.

101. Wang X, Zhao BS, Roundtree IA, Lu Z, Han D, Ma H, Weng X, Chen K, Shi H, He C. N(6)-methyladenosine Modulates Messenger RNA Translation Efficiency. Cell. 2015; 161(6): 1388–99.

102. Zhao BS, Wang X, Beadell A V, Lu Z, Shi H, Kuuspalu A, Ho RK, He C. m(6)A-dependent maternal mRNA clearance facilitates zebrafish maternal-to-zygotic transition. Nature. 2017; 542(7642): 475–8.

103. Ivanova I, Much C, Di Giacomo M, Azzi C, Morgan M, Moreira PN, Monahan J, Carrieri C, Enright AJ, O’Carroll D. The RNA m(6)A Reader YTHDF2 Is Essential for the Post-transcriptional Regulation of the Maternal Transcripote and Oocyte Competence. Mol Cell. 2017; 67(6): 1059–1067.e4.

104. Cai M, Liu Q, Jiang Q, Wu R, Wang X, Wang Y. Loss of m(6)A on FAM134B promotes adipogenesis in porcine adipocytes through m(6)A-YTHDF2-dependent way. JUBMB Life. 2019; 71(5): 580–6.

105. Zhong X, Yu J, Frazier K, Weng X, Li Y, Cham CM, Dolan K, Zhu X, Hubert N, Tao Y, Lin F, Martinez-Guryn K, Huang Y, Wang T, Liu J, He C, Chang EB, Leone V. Circadian Clock Regulation of Hepatic Lipid Metabolism by Modulation of m(6)A mRNA Methylation. Cell Rep. 2018; 25(7): 1816–1828.e4.

106. Wu R, Liu Y, Yao Y, Zhao Y, Bi Z, Jiang Q, Liu Q, Cai M, Wang F, Wang Y, Wang X. FTO regulates adipogenesis by controlling cell cycle progression via m(6)A-YTHDF2 dependent mechanism. Biochim Biophys acta Mol cell Biol lipids. 2018; 1863(10): 1323–30.

107. Li Z, Qian P, Shao W, Shi H, He XC, Gogol M, Yu Z, Wang Y, Qi M, Zhu Y, Perry JM, Zhang K, Tao F, Zhou K, Hu D, Han Y, Zhao C, Alexander R, Xu H, Chen S, Peak A, Hall K, Peterson M, Perera A, Haug JS, Parmely T, Li H, Shen B, Zeitlinger J, He C, Li L. Suppression of m(6)A reader Ythdf2 promotes hematopoietic stem cell expansion. Cell Res. 2018; 28(9): 904–17.

108. Li M, Zhao X, Wang W, Shi H, Pan Q, Lu Z, Perez SP, Suganther R, He C, Bjoras M, Kunglland A. Ythdf2-mediated m(6)A mRNA clearance modulates neural development in mice. Genome Biol. 2018; 19(1): 69.

109. Yu J, Li Y, Wang T, Zhong X. Modification of N6-methyladenosine RNA methylation on heat shock protein expression. PLoS One. 2018; 13(6): e0198604.

110. Winkler R, Gillis E, Lasman L, Safra M, Geula S, Soyris C, Nachshon A, Tai-Schmiedel J, Friedman N, Le-Trilling VTK, Trilling M, Mandelboim M, Hanna JH, Schwartz S, Stern-Ginossar N. m(6)A modification controls the innate immune response to infection by targeting type I interferons. Nat Immunol. 2019; 20(2): 173–82.

111. Toro-Ascuy D, Rojas-Araya B, Valiente-Echeverria F, Soto-Rijo R. Interactions between the HIV-1 Unspliced mRNA and Host mRNA Decay Machinery. Viruses. 2016; 8(11): 320.

112. Tirumuru N, Zhao BS, Lu W, Lu Z, He C, Wu L. N(6)-methyladenosine of HIV-1 RNA regulates viral infection and HIV-1 Gag protein expression. Elife. 2016; 5: e15528.

113. Tsai K, Courtney DG, Cullen BR. Addition of m6A to SV40 late mRNAs enhances viral structural gene expression and replication. PLoS Pathog. 2018; 14(2): e1006919.

114. Zhong L, Liao D, Zhang M, Zeng C, Li X, Zhang R, Ma H, Kang T. YTHDF2 suppresses cell proliferation and growth via destabilizing the EGFR mRNA in hepatocellular carcinoma. Cancer Lett. 2019; 442252–61.

115. Li J, Meng S, Xu M, Wang S, He L, Xu X, Wang X, Xie L. Downregulation of N(6)-methyladenosine binding YTHDF2 protein mediated by miR-493-3p suppresses prostate cancer by elevating N(6)-methyladenosine levels. Oncotarget. 2018; 9(3): 3752–64.

116. Shi Y, Di Giammartino DC, Taylor D, Sarkeshik A, Rice WJ, Yates JR 3rd, Frank J, Manley JL. Molecular architecture of the human pre-mRNA 3’ processing complex. Mol Cell. 2009; 33(3): 365–76.

117. Al-Maghrebi M, Brule H, Padkina M, Allen C, Holmes WM, Zehner ZE. The 3’ untranslated region of human vimentin mRNA interacts with protein complexes containing eEF-1gamma and HAX-1. Nucleic Acids Res. 2002; 30(23): 5017–28.

118. Pelaseyed T, Bretscher A. Regulation of actin-based apical structures on epithelial cells. J Cell Sci. 2018; 131(20): jcs221853.
119. Batchelor CL, Woodward AM, Crouch DH. Nuclear ERM (ezrin, radixin, moesin) proteins: regulation by cell density and nuclear import. Exp Cell Res. 2004; 296(2): 208–22.

120. Statello L, Maugeri M, Garre E, Nawaz M, Wahlgren J, Papadimitriou A, Lundqvist C, Lindfors L, Collen A, Sunnerhagen P, Ragusa M, Purrello M, Di Pietro C, Tigue N, Valadi H. Identification of RNA-binding proteins in exosomes capable of interacting with different types of RNA: RBP-facilitated transport of RNAs into exosomes. PLoS One. 2018; 13(4): e0195969.

121. Serpinskaya AS, Tuphile K, Rabinow L, Gelfand VI. Protein kinase Darkener of apricot and its substrate EF1gamma regulate organelle transport along microtubules. J Cell Sci. 2014; 127(Pt 1): 33–9.

122. Esposito AM, Kinzy TG. The eukaryotic translation elongation Factor 1Bgamma has a non-guanine nucleotide exchange factor role in protein metabolism. J Biol Chem. 2010; 285(49): 37995–8004.

123. Kim S, Kellner J, Lee C-H, Coulombe P A. Interaction between the keratin cytoskeleton and eEF1Bgamma affects protein synthesis in epithelial cells. Nat Struct Mol Biol. 2007; 14(10): 982–3.

124. Cho D-I, Oak M-H, Yang H-J, Choi H-K, Janssen GMC, Kim K-M. Direct and biochemical interaction between dopamine D3 receptor and elongation factor-1Bbetagamma. Life Sci. 2003; 73(23): 2991–3004.

125. Chu H, Chen Y, Yuan Q, Hua Q, Zhang X, Wang M, Tong N, Zhang W, Chen J, Zhang Z. The HOTAIR, PRNCR1 and POLR2E polymorphisms are associated with cancer risk: a meta-analysis. Oncotarget. 2017; 8(26): 43271–83.

126. Spehalski E, Capper KM, Smith CJ, Morgan MJ, Dinkelmann M, Buis J, Sekiguchi JM, Ferguson DO. MRE11 Promotes Tumorigenesis by Facilitating Resistance to Oncogene-Induced Replication Stress. Cancer Res. 2017; 77(19): 5327–38.

127. Li N, Kong J, Lin Z, Yang Y, Jin T, Xu M, Sun J, Chen L. Ezrin promotes breast cancer progression by modulating AKT signals. Br J Cancer. 2019; 120(7): 703–13.

128. D’Arcy BM, Swingle MR, Papke CM, Abney KA, Bouska ES, Prakash A, Honkanen RE. The Antitumor Drug LB-100 Is a Catalytic Inhibitor of Protein Phosphatase 2A (PPP2CA) and 5 (PPP5C) Coordinating with the Active-Site Catalytic Metals in PPP5C. Mol Cancer Ther. 2019; 18(3): 556–66.

129. Gouble A, Grazide S, Meggetto F, Mercier P, Delsol G, Morello D. A new player in oncogenesis: AUFI/hnRNPD overexpression leads to tumorigenesis in transgenic mice. Cancer Res. 2002; 62(5): 1489–95.

130. Li Y, Bakke J, Finkelstein D, Zeng H, Wu J, Chen T. HNRNP1 is required for rhabdomyosarcoma cell growth and survival. Oncogenesis. 2018; 7(1): 9.

131. Shi J, Liu H, Yao F, Zhong C, Zhao H. Upregulation of mediator MED23 in non-small-cell lung cancer promotes the growth, migration, and metastasis of cancer cells. Tumour Biol. 2014; 35(12): 12005–13.

132. Chung FF-L, Tan PFTM, Raja VJ, Tan B-S, Lim K-H, Kam T-S, Hii L-W, Tan SH, See S-J, Tan Y-F, Wong L-Z, Yam WK, Mai CW, Bradshaw TD, Leong C-O. Jerantine A induces tumor-specific cell death through modulation of splicing factor 3b subunit 1 (SF3B1). Sci Rep. 2017; 742504.

133. Hassaan MK, Kumar D, Naik M, Dixit M. The expression profile and prognostic significance of eukaryotic translation elongation factors in different cancers. PLoS One. 2018; 13(1): e0191377.

134. Zhang Z, Pan L, Ding Y, Lv J, Zhou P, Fang Y, Liu X, Zhang Y, Wang Y. eEF1G interaction with foot-and-mouth disease virus nonstructural protein 2B: Identification by yeast two-hybrid system. Microb Pathog. 2017; 112111–6.

135. Sammaibashi S, Yamayoshi S, Kawaoka Y. Strain-Specific Contribution of Eukaryotic Elongation Factor 1 Gamma to the Translation of Influenza A Virus Proteins. Front Microbiol. 2018; 91446.

136. Sasvari Z, Iztova L, Kinzy TG, Nagy PD. Synergistic roles of eukaryotic translation elongation factors 1Bgamma and 1A in stimulation of tombusvirus minus-strand synthesis. PLoS Pathog. 2011; 7(12): e1002438.
Аналіз інтерактому eEF1Bγ в ядерній фракції клітин аденокарциноми легені людини A549
Л. М. Капустян, І. Л. Лисецький, Т. В. Бондарчук, О. В. Новосильна, Б. С. Негруцький

Мета. Виявити нові функції фактора елонгації трансляції eEF1Bgamma (eEF1Bγ) в ядерній фракції клітин карциноми легені людини A549.

Методи. Білкі-партнери eEF1Bγ у ядерній фракції клітин аденокарциноми легені людини A549 були ідентифіковані за допомогою ко-імунопреципітації із наступною рідинною хроматографією та тандемною мас-спектрометрією (LC-MS/MS). Білкові мережі, до яких входить локалізований у ядрі білок eEF1Bγ, визначали за допомогою програми Cytoscape 3.2.0 із плагіном MCODE. Додатковий аналіз партнерів ядерного eEF1Bγ проводили за допомогою бази даних Mapofthecell.

Результати. Ідентифіковано 234 білки, що взаємодіють із eEF1Bγ в ядерній фракції клітин A549. Мережі білок-білокових взаємодій, до яких за- лучені ці білки, були проаналізовані за допомогою двох біоінформатичних підходів.

Висновки. Висунуто предпослідження, що сплайсинг пре-мРНК та регуляція стабільності мРНК можуть бути основними процесами, у яких бере участь ядерно локалізований пре-мРНК. При цьому регуляція кількості специфічних мРНК через контроль сплайсингу відповідних пре-мРНК та вплив на стабільність мРНК.

Ключові слова: eEF1Bγ, білок-білокові взаємодії, ядро, клітини A549.

Analysis of eEF1Bγ interactome in the nuclear fraction of A549 human lung adenocarcinoma cells

Л. М. Капустян, И. Л. Лисецкий, Т. В. Бондарчук, А. В. Новосильная, Б. С. Негруцкий

Цель. Выявить новые функции фактора элонгации трансляции eEF1Bγ в ядерной фракции клеток карциномы легкого человека A549.

Методы. Белки-партнеры eEF1Bγ в ядерной фракции клеток аденооарциному легкого человека A549 были идентифицированы с помощью ко-иммунопреципитации с последующей жидкосной хроматографией и тандемной масс-спектрометрией (LC-MS/MS). Белковые сети, в состав которых входят локализованный в ядре eEF1Bγ, определяли с помощью программы Cytoscape 3.2.0 с плагином MCODE. Дополнительный анализ партнеров ядерного eEF1Bγ проводили, используя базу данных Mapofthecell.

Результаты. Идентифицированы 234 белка, которые взаимодействуют с eEF1Bγ в ядерной фракции клеток A549. Сети белок-белковых взаимодействий, в которых участвуют данные белки, были проанализированы с помощью двух биоинформационных подходов.

Выводы. Выдвинуто предположение, что сплайсинг пре-мРНК и регуляция стабильности зрелой мРНК могут быть основными процессами, в которых участвует локализованный в ядре пре-мРНК. Во время карциногенеза, некоторая часть молекул eEF1Bγ заливаются в ядро, где регулирует количество специфических мРНК посредством контроля сплайсинга соответствующих пре-мРНК и влияния на их стабильность.

Ключевые слова: eEF1Bγ, белок-белковые взаимодействия, ядро, клетки A549.

Received 15.04.2019