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eIF4A1 is Associated With TrkA and Inhibits TrkA Polyubiquitination*

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Abstract Nerve growth factor (NGF) can bind to cell surface receptor p75NTR and TrkA and play a vital role in cell differentiation, survival, apoptosis, proliferation and migration. TrkA interacted with multiple proteins *in vivo*, but due to the complexity of the NGF signaling pathway, it is still necessary to explore more proteins that interact with TrkA to gain a more accurate understanding of the NGF pathway. Here we report eIF4A1 is a new partner of TrkA. We found eIF4A1 interacted with TrkA *via* the yeast two hybrid assay. And then the association between TrkA and eIF4A1 was identified by GST pull-down and co-immunoprecipitation assay. Additionally, NGF stimulated this interaction and the associated binding domain is the N-terminus domain (NTD) in eIF4A1 and the TK domain in TrkA. And eIF4A1 could colocalize with TrkA at cell membrane. Furthermore, eIF4A1 also inhibits TrkA polyubiquitination through lysine (Lys)-63-linked polyubiquitin chains which causes its internalization. So eIF4A1 plays a novel role in NGF signaling pathway.

Key words TrkA, eIF4A1, interaction, ubiquitination
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Neurotrophins are the most well-known trophic factors in mammalian nervous system. Its members containing nerve growth factor(NGF), brain-derived neurotrophic factor(BDNF), neurotrophin-3(NT-3), and neurotrophin-4/5(NT-4/5) derived from a common ancestral gene. And their sequence and structure share high similarity and they are all collectively called neurotrophins[1]. Neurotrophins have extensive functions that regulate development, survival and removal of normal cell in the nervous system, affect cell proliferation, differentiation in cancer. In addition, neurotrophins also mediate some senior neurological activities such as learning and memory enhancement, and behavioral plasticity[2-3].

Neurotrophins transmit signals into cells mainly through two types of membrane receptors, the p75 neurotrophin receptor (p75NTR) and the tropomyosin-related kinase (Trk). p75NTR is a glycoprotein which can bind to each neurotrophic compound with equal affinity, but the affinity is lower[4]. In contrast to p75NTR, the Trk family also includes three types, TrkA, TrkB and TrkC. Among the Trk receptor, NGF specifically binds to TrkA, BDNF and NT-4 specifically bind to TrkB, while NT-3 specifically binds to TrkC[5]. The p75NTR and Trk receptor are low-affinity and high-affinity receptors for neurotrophins, respectively. The tyrosine kinase receptor, Trk, its endogenous kinase activation needs ligand-dependent binding. Different neurotrpphin bind different Trk receptor to perform different functions. In recent years, some in-depth studies have been conducted on the signaling pathway of the TrkA receptor. Polyubiquitination of TrkA directs its

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internalization and the cascade signaling[6]. NTRK gene fusions often occurs in cancers especially glioma[7]. The high expression of TrkA in HER2-negative parotid ductal carcinoma (SDC) was significantly associated with poor overall survival (OS) and the gene TrkA in clinical research may be a promising therapeutic target in SDC, especially in HER2-negative SDC[8].

Eukaryotic translation progress required many factors. Protein synthesis initiated with binding to the eukaryotic translation initiation factor(eIF)4F which can recruit the ribosomes binding to mRNA and then initiates the translation process of mRNA to protein. eIF4F consists of three subunits, the eIF4E cap binding protein, the eIF4A RNA helicase, and the eIF4G scaffold protein. eIF4A has RNA-dependent ATPase activity and ATP-activated RNA-binding activity, and its helicase activity is thought to be related to the opening of the secondary structure of the 5'-end non-coding sequence of mRNA for the recruitment of ribosomes. eIF4A is classified into a free type and a complex type depending on whether it binds to a ribosome, and the former activity is only 1/20 of the latter. There are three members of the eIF4A family, eIF4A1, eIF4A2 and eIF4A3, which belong to a family of helicase proteins called DEAD-box. In addition to interact with components involved in translation initiation complexes, eIF4A is also a target for inhibiting translation initiation, such as Pdcd4[9], Patemine A[10], 15d-PGJ2[11] and BC1 RNA[12].

In our previous work, it was confirmed that p75NTR/TrkA can interact with RanBPM, and the TrkA intracellular region was identified by immunoprecipitation assay. In present work, we show TrkA interacts with eIF4A1 and we attempt to study the high complexity and precision of protein expression regulation in eukaryotic cells. The structural basis for the interaction between TrkA and eIF4A1 and its effects on protein translation will be explored at the level of translation initiation complex.

1 Methods

1.1 Yeast two hybrid screening

The TrkA-ICD expressing yeast cells Y190 were obtained by using a high-efficiency LiAC transformation method with plasmid pAS-TrkA-ICD. Then the yeast cells Y190-TrkA-ICD were transformed with a Matchmaker two-hybrid library containing cDNA from mouse brain. Yeast cells were spread on synthetic dropout (SD) plates (Leu^-, Trp^-, His^-, β-gal). After about 4 – 6 days of growth, several positive colonies were observed. The blue ones were picked up and then replated on SD plate (Leu^-, Trp^-, His^-, Ura^-). Transformants that met the two criteria of growth in the absence of Ura and successful activation of β -galactosidase were supposed to be positive interactions.

1.2 Cell culture, transfection and NGF stimulation

HEK 293T cell line in experiment was ordered from Cell Resource Center from the Institute of Basic Medical Sciences, Chinese Academy of Medical Sciences and the cells were incubated in DMEM (high glucose) with 10% FBS in a 5% CO_2 humidified incubator at 37℃. After cell confluence reaches 90%, we performed the cell transfections by using Lipofectamine 3 000 (Invitrogen) following the manufacturer's instructions. Transfected cells were continued to be cultured in complete medium. For NGF stimulation, firstly the cells were shifted to 3% serum medium for at least 3 h. After starvation, NGF (final concentration is 100 μg /L) was added for the indicated time.

1.3 Plasmids, antibodies and reagents

The complete coding sequences of TrkA and eIF4A1 were obtained by Reverse Transcriptase-Polymerase Chain Reaction (RT-PCR). Total RNA was extracted from HEK 293T cells. The cDNA was synthesized using the first strand cDNA synthesis kit (GoScript Reverse Transcription System, Promega). The genes were amplified and the products were constructed into the vector of pCMV-HA or pCMV-Myc, respectively. And then TrkA, as a template, its intracellular domain (TrkA-ICD) was cloned in frame with the pAS2-1 vector containing the GAL4 DNA binding domain, or the plasmid pCMV-HA. GST tagged truncations of TrkA and eIF4A1 were obtained by PCR amplifying the corresponding coding sequence. Consequently, the PCR products were cloned into respective vectors. All of the constructed plasmids were sequenced and confirmed correct. The mentioned vectors above we constructed are named as pGEX-4T-2-eIF4A1, pGEX-4T-2-eIF4A1-NTD, pGEX-4T-2-eIF4A1-CTD, pGEX-4T-2-TrkA-TK, pGEX-4T-2-TrkA-ATP, pGEX-4T-2-TrkA-JM and

pFlag-Ub were kept in our laboratory.

Mouse anti- β -actin (A5441, 1∶ 5 000) and mouse anti-HA antibody (H9658, 1∶ 2 000) were used and purchased from Sigma Aldrich (St Louis, MO, USA). Mouse anti-c-Myc (SC-40, 1∶2000 and 1∶ 50 for IF) and rabbit anti-TrkA (SC-118, 1∶ 1 000) were used and from Santa Cruz (Santa Cruz, CA, USA). Plasmid DNA extraction maxi kit and purification kit were bought from Vigorous Biotechnology (Beijing, China). Lipofectamine 3000™ was ordered from Thermal Scientific (Carlsbad, CA, USA).

1.4 GST pull−down assay

GST-TrkA-ICD, GST-eIF4A1 and their series of deletion mutants were purified in *E. coli* as previously described[13]. TrkA-ICD was transcribed/ translated with TNT Quick Coupled Transcription /Translation Systems to produce a TrkA protein *in vitro*. The purified GST fusion protein or control GST protein was incubated together with lysates from HEK 293T cells which were transfected with HA-TrkA-ICD, or Myc-eIF4A1, or TrkA-ICD translated *in vitro*. After incubation overnight, the beads were clarified by centrifugation for 5 min at 500 *g* and washed with cold lysis buffer two times and suspend in SDS loading buffer at 4℃. The bound protein was checked by Western blotting probed with indicated antibody.

1.5 Co−immunoprecipitation and immunoblotting

HEK 293T cells, grown in 6-well plate, were lysed with cold lysis buffer containing 50 mmol/L Tris-HCl (pH 7.5), 1% Triton X-100, 150 mmol/L NaCl and 10% glycerol with protease inhibitors. After about 15 min incubation at 4℃ followed by centrifugation at 10 000 *g* for 10 min, supernatants were transferred to another Eppendorf tube and the concentration was measured using Bradford assay. About 1 mg total protein was used for the immunoprecipitation. First, the lysates were pre-cleared with the protein A-Sepharose and mouse IgG for 1 h at 4℃. Then the lysates were centrifuged and the pellet was discarded. The clarified supernatants were added to 1 μg anti-HA or anti-Myc antibody and 30 μl protein A-Sepharose at 4℃ overnight. Then the lysates were centrifuged at 1 000 *g* for 5 min and washed with cold lysis buffer for three times.

Then bound proteins were diluted with 2×SDS-PAGE loading buffer and denatured in boiled water bath, separated on 12% SDS-PAGE, and then transferred onto nitrocellulose membrane. After blocking with 5% milk in TBS/T (0.1% Tween-20 in TBS buffer), Western blotting was performed to detect the target protein using the appropriate primary antibodies. Washing with TBS/T three times, the membrane was incubated with HRP-conjugated secondary antibodies. And then the membrane was detected with pierce ECL Western blotting substrate and exposed to X-ray film.

1.6 Immunofluorescence assay

HEK 293T cells were cultured, digested with 0.05% Trypsin-EDTA solution and grown on coverslips. The cells on coverslips were transfected with Myc-eIF4A1 and HA-TrkA. After transfection for 24 h, HEK 293T cells were fixed with 4% Paraformaldehyde. After washing, cells were permeated with 0.1% Triton X-100 for 30 min and then incubated with rabbit anti-TrkA antibody and mouse anti-Myc antibody. As the secondary antibody, goat anti-mouse labelled with TRITC for detecting eIF4A1 and goat anti-Rabbit labelled with FITC for detecting TrkA were used. After washing with PBS/T (0.1% Tween-20 in TBS buffer), cellular nuclei were counterstained with DAPI. Slides were photographed using a Leica TCS SP5 microscope.

1.7 Cytoplasmic and nuclear extraction

HEK 293T cells were transfected with HA-TrkA, Myc-eIF4A1 or HA-TrkA and Myc-eIF4A1. At 48 h after transfections, cytoplasmic and nuclear fractions were extracted as previously described[14]. And then Western blotting was performed using the indicated antibodies.

2 Results

2.1 Identification of the novel association of TrkA and eIF4A1

To find the new TrkA interacting proteins, yeast two hybrid assay was performed to screen a mouse cDNA library. The TrkA intracellular domain (ICD) as a bait was constructed into plasmid pAS2-1 framing with its DNA Binding (DB) domain. After selected on SD/-Leu/-Trp medium, several positive clones were picked out among the cDNA library and these insertions were sequenced. Subsequently, we compared this inserted sequence with the Genbank database using BLAST and we confirmed two interacting partners, Snapin and eIF4A1. And then the TrkA-eIF4A1 association was further verified by

yeast two-hybrid using pAS-TrkA-ICD and pACT-eIF4A1, a prey plasmid in which eIF4A1 was framed with GAL4 activation domain (AD) in plasmid pACT2 (Figure 1a). Three days after transformation, the recombinant clones co-transformed with the pAS-TrkA-ICD and pACT-eIF4A1 had outgrown on SD/-Ura/-His/-Leu/-Trp as same as the positive control (Figure 1b). Additional β -galactosidase assay was used to verify the specific interaction between TrkA and eIF4A1 (Figure 1c). These preliminary experimental results manifested that TrkA-ICD physically interacts with eIF4A1.

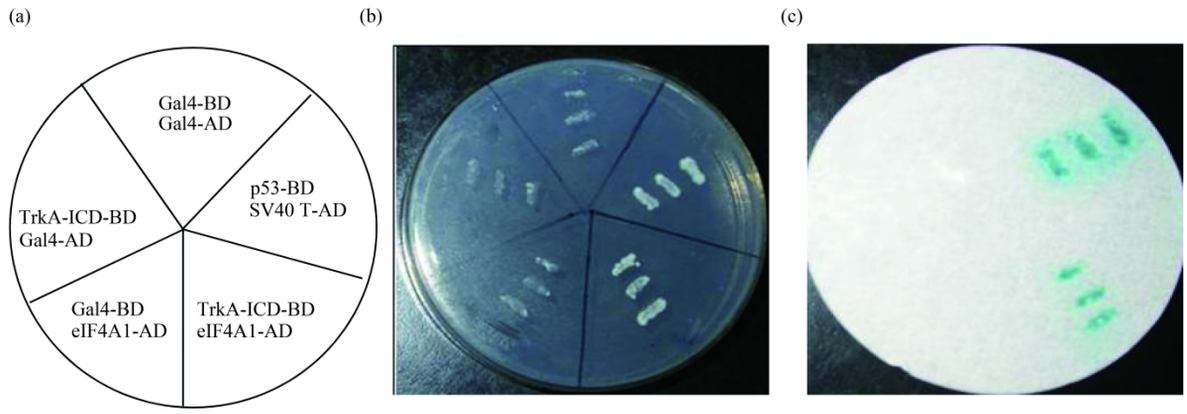

Fig. 1 Identification of the novel association of eIF4A1 and TrkA

（a）Schematic summary of yeast strain Y190 containing the indicated BD plasmids co-transformed with AD plasmids.（b）Growth of transformed yeast cells containing different constructs was compared by streaking onto SD/-Ura/-His/-Leu/-Trp plate.（c）Activity of β-galactosidase in the LacZ assay.

2.2 eIF4A1 binds to TrkA−ICD *in vivo* and *in vitro*

Intending to verify the association between TrkA-ICD and eIF4A1, we applied to glutathione S-transferase (GST) pull-down experiments using recombinant GST-eIF4A1. GST-eIF4A1 fusion protein and control GST proteins were affinity purified on glutathione-Sepharose 4B and the proteins immobilized on beads were stained with the Coomassie Brilliant blue (Figure 2a, up panel). Subsequently, the immobilized beads were incubated with equal dosage of lysates which were transfected with pHA-TrkA-ICD. Compared with GST line, the GST-eIF4A1 fusion protein could precipitate with HA-TrkA-ICD, implying that TrkA interacts with eIF4A1 *in vitro* (Figure 2a, down panel).

And then to obtain direct evidence that TrkA binds to eIF4A1, we performed a *vitro* binding assay by using TNT Quick Coupled Transcription / Translation Systems to produce a TrkA protein *in vitro*. After incubation with the GST or GST-eIF4A1, the pull-down beads were dissolved in SDS loading buffer and were analyzed by Western blotting using anti-TrkA antibody (Figure 2b). Collectively, TrkA directly bound recombinant GST-eIF4A1.

In addition, we wanted to determine whether TrkA could interact with eIF4A1 in mammalian cells. First, HEK 293T cells were co-transfected using the plasmid encoding tagged TrkA-ICD and eIF4A1, respectively. The following reciprocal co-immunoprecipitation (Co-IP) assay was performed using anti-Myc or anti-HA antibody to validate the interaction of TrkA and eIF4A1. And the immunoprecipitated complex was detected by immunoblotting. The results demonstrated that after immunoprecipitation of HA-TrkA-ICD using anti-HA antibody, we found Myc-eIF4A1 was in the immune complex, and in turn HA-TrkA-ICD was in the immunoprecipiation complex by anti-Myc antibody (Figure 2c). In conclusion, we demonstrated that the association between TrkA and eIF4A1 exists *in vitro* and *in vivo*.

To identify whether the interaction between TrkA and eIF4A1 was respond to NGF stimulation, 293T cells were co-transfected with plasmids encoding full length TrkA and eIF4A1 and followed by NGF treatment. The Co-IP result suggested that the bound TrkA to eIF4A1 was increased under stimulation of

NGF for the indicated time period when the cells was co-transfected with the HA tagged full length TrkA and Myc-eIF4A1 into cells (Figure 2d). Taken together, we conclude that eIF4A1 interacts with TrkA.

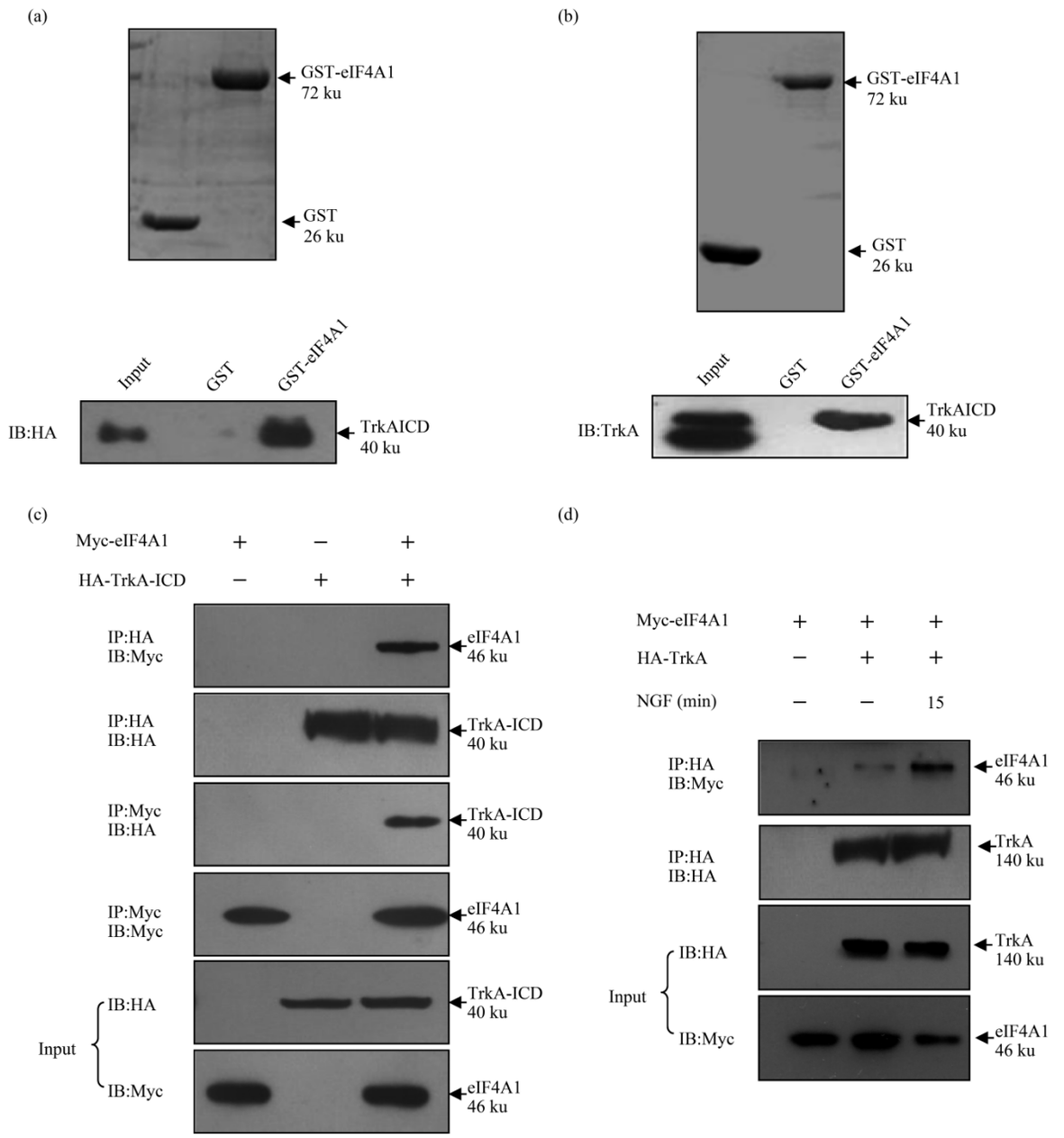

Fig. 2　eIF4A1 binds to TrkA *in vivo* and *in vitro*

（a） SDS-PAGE and Coomassie bright blue staining of recombinant GST or GST- eIF4A1 fusion proteins （Up panel）. The bound proteins were analyzed and immunoblotted with anti-HA antibody （Down panel）. （b） TrkA-ICD was transcribed *in vitro* by using TNT Quick Coupled Transcription /Translation Systems. The precipitated complex was detected using anti-TrkA antibody. （c） Immunoprecipitation was performed by using an anti-Myc or anti-HA antibody，followed by Western blotting with anti-Myc or anti-HA antibodies. （d） Proteins immunoprecipitated with anti-HA antibodies were subjected to immunoblotting with antibodies against anti-Myc or anti-HA.

2.3 Mapping the interacting domain between TrkA and eIF4A1

To study the detailed binding domain responsible for the linkage between TrkA and eIF4A1, the GST pull-down assay was applied. Three truncation domains for TrkA and two for eIF4A1 were constructed as diagrammed in Figure 3a. The GST pull-down experiments showed that only the eIF4A1-NTD remains the interaction with TrkA-ICD (Figure 3b). And TK domain in TrkA was indispensable for the association with eIF4A1 (Figure 3c). These results indicate that the interaction between TrkA and eIF4A1 is *via* the eIF4A1-NTD and the TK domain in TrkA.

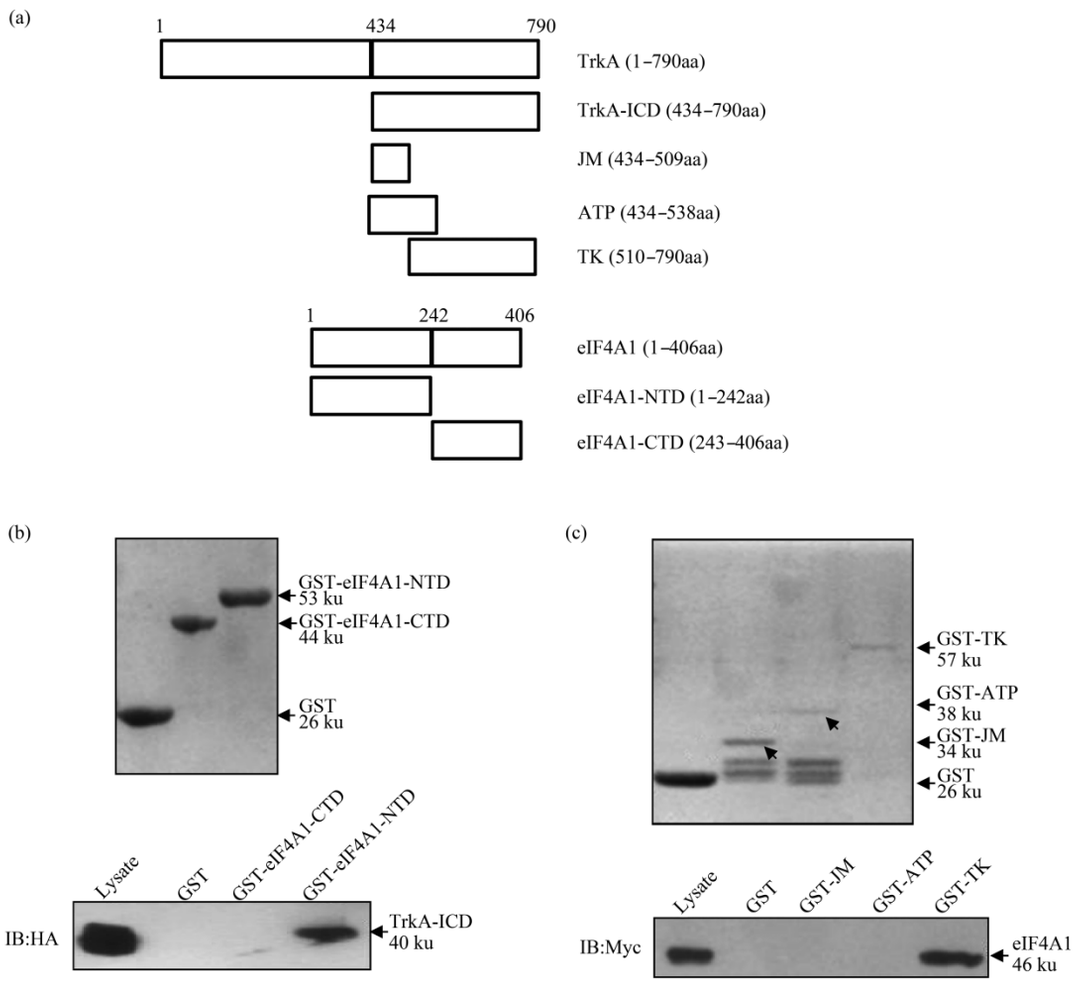

Fig. 3 Mapping the interacting domain between TrkA and eIF4A1

（a）Schematic structure of TrkA and eIF4A1.（b）The precipitated proteins were analyzed by immunoblotting with an anti-HA antibody.（c）The bound proteins were analyzed by immunoblotting with an anti-Myc antibody.

2.4 Membrane co−localization of TrkA and eIF4A1

To further investigate the association between TrkA and eIF4A1 at cellular level, Myc-eIF4A1 and HA-TrkA ectopically expressed in HEK 293T cells. After transfections, immunofluorescence was performed to visualize the distribution of eIF4A1 and TrkA. As seen in Figure 4a, TrkA was predominantly located in cell membrane and cytoplasm. And eIF4A1 was mostly located in the cytoplasm. Moreover, when TrkA was co-transfected with eIF4A1, the merged image showed that the ectopic expression of proteins apparently colocalized at plasma membrane.

To confirm this result, transfected 293T cells were fractionated into cytoplasmic and unclear constituents, and the subcellular distributions were

detected with immunoblotting. The purity of fractions was checked by immunoblotting with anti-Lamin A/C and β -tubulin, respectively. Consistent with the previous immunofluorescence assay, transfected Myc-eIF4A1, HA-TrkA or Myc-eIF4A1 and HA-TrkA all resulted in cytoplasmic accumulation (Figure 4b). It is likely that eIF4A1 interacts with TrkA at cell membrane.

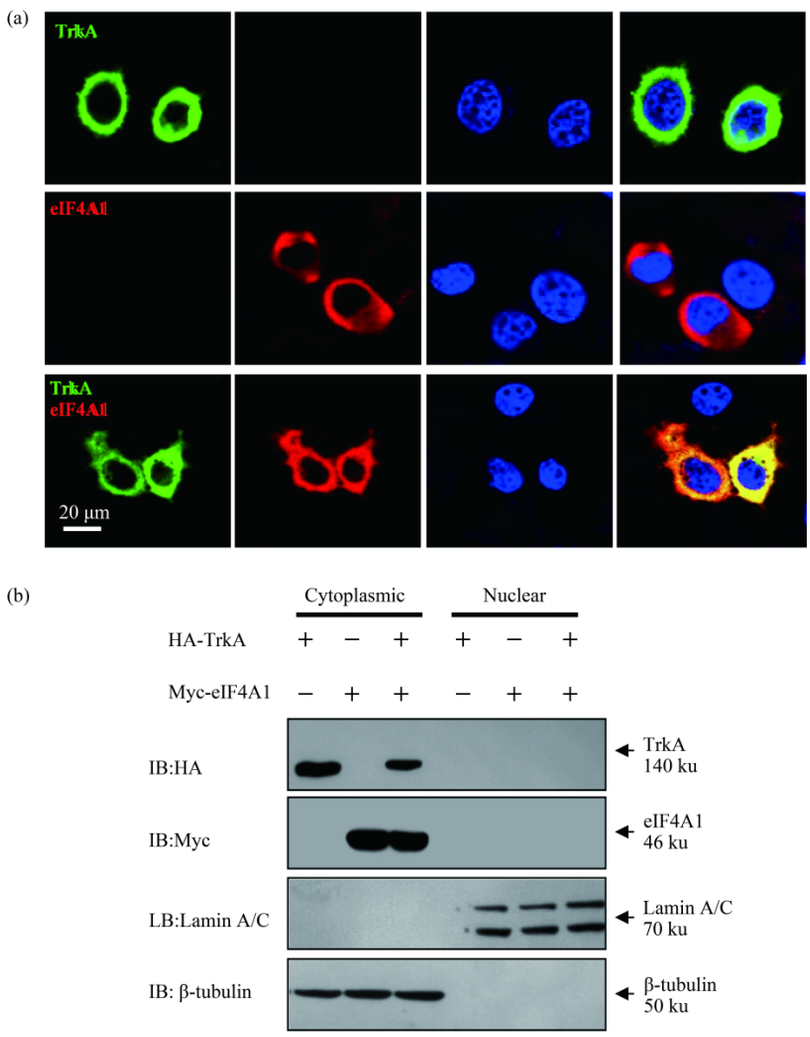

Fig. 4　Membrane co−localization of TrkA and eIF4A1

（a） Representative confocal microscopy pictures of 293T cells ectopically expressing HA-TrkA and Myc-eIF4A1. The cells were stained with anti-Myc antibody （TRITC，red color） and anti-TrkA antibody （FITC， green color）. Staining with DAPI （blue color） was used to visualize the cell nuclei. （b） Fractionation of 293T cells extracts. 293T cells were transfected with the indicated plasmids. 24 h later，cells were fractionated into cytoplasmic and nuclear composition. The target protein were analyzed by immunoblotting with anti-HA or anti-Myc antibody. The anti-Lamin A/C or anti-β-tubulin antibody was used to confirm nuclear or cytoplasmic fraction.

2.5　NGF stimulated TrkA Lys 63−linked polyubiquitination and eIF4A1 repressed TrkA polyubiquitination

NGF can evoke Lysine 63 polyubiquitination of its receptor TrkA and regulate TrkA internalization and signaling[6]. HA tagged TrkA was transfected into 293T cells along with a Myc-Ub mutant containing one lysine only at position 48 (K48-0nly) or at position 63 (K63-only). And transfection for 24 h, the cells were starved and stimulated with NGF for 30 min. Then the transfected cells were lysed. Subsequently, immunoprecipitation assay was performed to check the ubiquitination of TrkA and the expression of target protein. The result demonstrated

that NGF evokes elevation of K63-only ubiquitin of TrkA (compare lane *2* with *1*), not K48-only ubiquitin in Figure 5a (compare lane *4* with *3*).

To analyzing the biological role of eIF4A1 in TrkA ubiquitination, 293T cells were transfected with different combinations of plasmids as indicated in the Figure 5b, and then were treated or untreated with NGF. We observed that Myc-eIF4A1 could prevent TrkA polyubiquitination stimulated with NGF (compare lane *4* with *2*) by immunoprecipitation analysis (Figure 5b). Thus, our data demonstrated that eIF4A1 repress NGF evoked TrkA polyubiquitination.

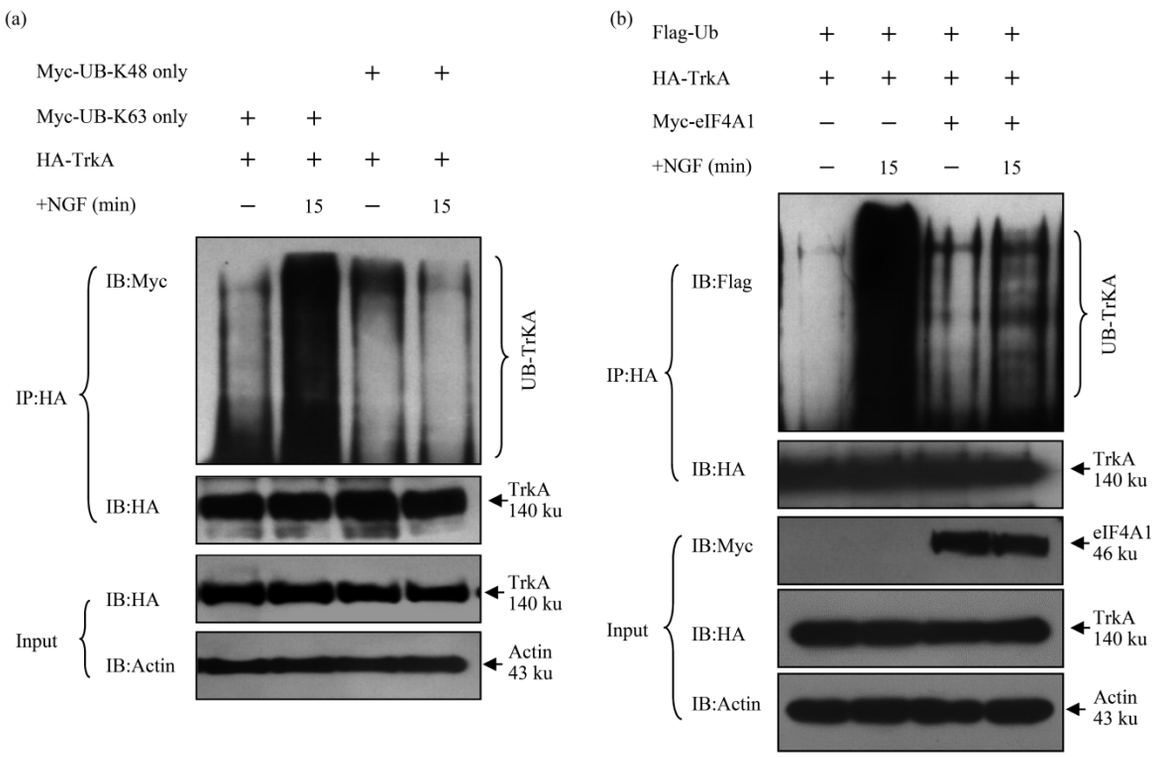

Fig. 5　NGF stimulated TrkA Lys 63−linked polyubiquitination and eIF4A1 repressed TrkA polyubiquitination

（a）NGF stimulated TrkA Lys 63-linked polyubiquitination. 293T cells were co-transfected with HA-TrkA and Myc tagged K63-only or K48-only ubiquitin. After transfection，cells were stimulated with 100 μg/L NGF for 30 min. Complex immunoprecipitated with anti-HA antibodies and was immunoblotted with anti-Myc antibody for detection of ubiquitinated TrkA. （b）eIF4A1 repressed NGF induced TrkA polyubiquitination. Ubiquitination level was checked by immuoprecipitation with anti-HA antibody and immunoblotted with an anti-Flag antibody.

3　Discussion

Our present work provides the first evidence of the modulation of NGF signaling by eIF4A1 through a mechanism involving a direct interaction between eIF4A1 and TrkA. To explore the new function of TrkA, we probed the possible interactions using yeast two-hybrid system screening method. Several positive proteins were identified and sequenced and then the interaction was confirmed *in vitro*, through a GST pull-down experiment and *in vivo* through the Co-IP assay. The N-terminus domain (NTD) of eIF4A was confirmed to interact with TrkA, and the TK of TrkA was responsible for binding to eIF4A1. Experiments with ectopically expressing TrkA and eIF4A1 in mammalian cell line verified this protein interaction previously found. Together, the *in vivo* and *in vitro* results clearly supported that TrkA specifically interacted with eIF4A1.

A novel function of eIF4A1, in which it could repress TrkA polyubiquitination evoked by NGF, was detected in this work and may regulate the NGF signaling. Receptor tyrosine kinases (RTKs) participate in vital signaling pathways that regulate cell differentiation, cell survival, proliferation and migration. Aberrantly activated RTKs had been linked to human cancers. Understanding the underlying

mechanisms has implications for cancer therapy. Post-translational protein modification was one of the important regulatory mechanism of cellular proteins with a number of biological functions[15]. To date, multiple protein modification have been identified, including phosphorylation, acetylation, ubiquitination and SUMOylation.

Ubiquitination is a wide studied method of post-translational modification that is related to mediate protein degradation, endocytosis, and the sorting, as well as protein-protein interaction and regulates many biological processes, including immune responses, apoptosis and cancer[16]. And dysregulation of the ubiquitin pathways lead to diseases including tumorigenesis [17]. Regulation of TrK receptor ubiquitination was vital for its internalization and degradation[18]. The ubiquitin ligases, Cbl family plays an important role in RTKs ubiquitination and mediating its degradation. c-cbl can promote ligand-induced ubiquitination and subsequent TrkA degradation[19]. Nevertheless, TRAF4 promoted TrkA ubiquitination modifications on its kinase domain. This alteration hyperactivated TrkA kinase activity and altered its phosphorylation status[20]. Deubiquitinating enzymes (DUBs) remove the ubiquitin from its conjugates. USP8 was recognized to be a DUB that could deubiquitinate TrkA *in vivo* and *in vitro*[21]. But, there was scarcely reporting that protein other than DUBs could inhibit the TrkA ubiquitination.

It was previously reported that NGF could promote protein synthesis through activation of eIF2B in PC12 cells[22]. NGF can induce phosphorylation of 70 ku ribosomal protein, S6 kinase, eIF4E-BP1, and eIF2B, and activate downstream signaling pathways to promote protein synthesis. But the precise regulating mechanism need to be elucidated that what the role of TrkA plays in this process. NGF could induce TrkA polyubiquitination and the subsequent its internalization. In our work, we found that the eIF4A repressed the NGF evoked internalization and TrkA polyubiquitination. eIF4A, which is a component of the eIF4F complex, its helicase activity is thought to be related to the opening of the secondary structure of the 5'-end non-coding sequence of mRNA for ribosome recruitment. In addition to participating in the translation start, eIF4A also played a vital role in various developmental processes. eIF4A was found to be a regulatory proteins of Decapentaplegic (Dpp) signaling pathway in *Drosophila* and further studies had shown that eIF4A regulates the Dpp signaling pathway by mediating the degradation of the Dpp signaling molecule Mad and Medea in translation-independent manner[23].

Our recent finding has revealed that TrkA and eIF4A1 could co-localize under NGF stimulation and repressing its polyubiquitination. And then the receptor signal switched on and the cell signal was able to resume. This is a useful attempt to study the high complexity and precision of protein expression regulation in eukaryotic cells. The structural basis for interaction between TrkA and eIF4A1 proteins and the effect on protein translation will be explored at the level of translation initiation complex.

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eIF4A1与TrkA相互作用后抑制TrkA的泛素化*

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摘要　神经生长因子（NGF）结合细胞表面受体p75NTR（p75神经营养素受体）和TrkA（酪氨酸蛋白激酶A）后介导了细胞分化、细胞生存、凋亡、增殖和侵袭等多个重要的生理病理过程. TrKA能与细胞内多个蛋白质相互作用，但是由于NGF信号通路的复杂性，现在仍有必要发现与之相互作用的蛋白质以更准确地了解NGF信号通路. 本研究中我们通过酵母双杂交的方法筛选到了一个新的与TrKA相互作用的蛋白质——真核生物翻译起始因子4A1（eIF4A1），然后通过谷胱甘肽巯基转移酶融合蛋白沉降实验（GST-pull-down）和免疫共沉淀实验（Co-IP）证明了TrkA和eIF4A1的相互作用. 此外NGF能够增强TrkA和eIF4A1的相互作用. 在鉴定相互作用位点实验中，我们发现eIF4A1的氨基端结构域和TrkA的TK结构域参与了相互作用. TrkA和eIF4A1共定位在细胞膜上. NGF能够引起TrkA与泛素蛋白63位的赖氨酸连接，而eIF4A1与TrkA相互作用后能够抑制TrkA与泛素蛋白63位的赖氨酸连接. 综上，得出结论 eIF4A1通过与TrkA相互作用抑制其泛素化调控NGF信号通路.

关键词　TrkA，eIF4A1，相互作用，泛素化

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