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Molecular Brain Research 134 (2005) 333–337
MOLECULAR BRAIN RESEARCH
www.elsevier.com/locate/molbrainres

Short communication

Local protein synthesis by BDNF is potentiated in hippocampal neurons exposed to ephrins

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Accepted 22 October 2004
Available online 15 December 2004

Abstract

Local protein synthesis in neuronal dendrites is one of the mechanisms that may mediate a rapid and synapse-specific mobilization of proteins from the resident mRNAs. A great deal of effort has been made in analyzing the dynamic state of protein synthesis in the living cells chiefly by quantifying protein level. However, the protein level cannot mirror the spatiotemporal alteration of translation because it can be affected, not only by protein synthesis, but also by other factors, like degradation. Therefore, it is problematic to visualize the dynamic state of translation by the present methods. To solve the problem, we applied fluorescence resonance energy transfer (FRET) technique to in situ detection of the assembly and disassembly cycle among a pair of translation initiation factors [eukaryotic initiation factors (eIFs)], thereby showing that BDNF and ephrin could potentiate local protein synthesis in the dendrites of hippocampal neurons.

Theme: Development and regeneration
Topic: Formation and specificity of synapse

Keywords: Local protein synthesis; Dendrite; eIFs; FRET; Ephrin-Eph signal; BDNF

Over the past two decades, a considerable number of studies have reported the regulated localization of specific mRNAs in the dendritic and/or axonal compartment [8,16,18]. The presence of components of translational machinery, such as rRNAs, endoplasmic reticulum (ER), Golgi apparatus, and translational factors [including eukaryotic initiation factors (eIFs)] in the dendrites raised the possibility of active translation from the localized mRNAs [7,21,23]. This local protein synthesis hypothesis has received increasing experimental supports [6,13,22]; however, it has not yet been demonstrated that the observed alteration of protein level in the dendrites is a pure result of a translational change. To elucidate how localized protein synthesis is regulated in the dendrites, we focused on the dynamic processes of translation itself rather than its product. One candidate is a translation initiation step, which comprises a cascade of interactions among eIFs at each round of ribosome entry into the initiation codon (Fig. 1A) [1,3,4]. We focused on a direct interaction between eIF2 and eIF5 that are made prior to 48S initiation complex formation (multifactor complex; Fig. 1A) and broken by eIF5-dependent GTP hydrolysis of eIF2 to displace the eIF complex with 60S ribosome (Fig. 1A). We attempted to visualize the interaction before and after mature 80S ribosome formation using fluorescence resonance energy transfer (FRET) technique [15] and to apply this method to detecting ongoing translation in the dendrites of hippocampal neurons that received treatment with BDNF and/or ephrin.

We prepared a FRET probe mimicking the behavior of the eIF2β/eIF5 (V2b-5C). As a negative control, we prepared the ΔN-eIF2β/eIF5 probe (VΔN-5C) which inhibits the

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doi:10.1016/j.molbrainres.2004.10.034

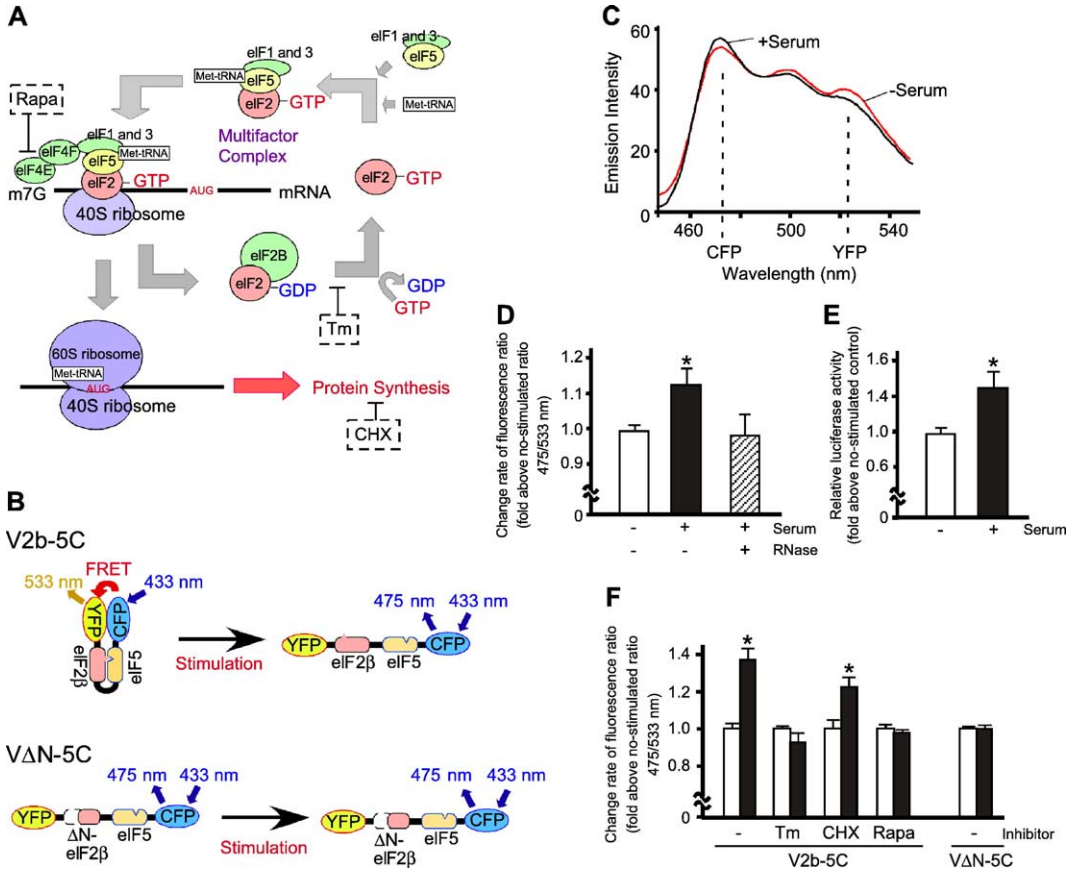

Fig. 1. A strategy for translation-sensitive FRET based on protein interactions among eIFs. (A) Overview of translation initiation reactions among eIFs and the pathways impaired by the translation inhibitors. We targeted two molecular interactions of eIF5 and eIF2β. (B) The schematic representations of the V2b-5C that imitates the native interactions among eIF2β and eIF5. A negative control, VΔN-5C, had no interaction between eIF2 and eIF5 moieties. When the intramolecular interaction brought each of the fluorophores closer to less than 10 nm, fluorescent energy from the excited CFP (Donor) with 433-nm fluorescence causes an 533-nm emission from the YFP color variant, Venus (Acceptor); otherwise, the probes cause a 475-nm emission from CFP itself. (C) Emission spectra of the extracts from the V2b-5C-expressing HEK293T. The cells were cultured in DMEM supplemented with 0.5% FBS (−) and stimulated with 20% FBS for 24 h (+). (D) Statistical analysis of emission ratios are shown in panel (C). The asterisk indicates a significant effect of 20% FBS on FRET efficiency (n=3 in each column; p<0.01). RNase A impaired this effect. (E) Induction of protein synthesis by the high-serum condition. HEK293T cells expressing luciferase mRNA were incubated under 0.5% or 10% serum-containing medium for 12 h. Even in the absence of actinomycin D, serum stimulation enhanced the luciferase activity. (F) Emission ratios of V2b-5C which were expressed in the NIH3T3 cells pretreated with various protein synthesis inhibitors, like cycloheximide (CHX; 10 μg/ml), tunicamycin (Tm; 50 μg/ml), and rapamycin (Rapa; 100 μM) for 12 h. Dark bars, ratios from serum-treated cells. White bars, ratios from nontreated cells. Asterisks indicate significant effects of 20% FBS on FRET efficiency even after protein synthesis inhibition (p<0.01). In panels (C), (D), and (E), error bars represent the standard deviation of the results from three independent experiments.

FRET-causing intramolecular interaction by deleting the N-terminal three lysine-rich motifs from the eIF2β moiety (1–132 a.a. deletion of K-box from eIF2β) (Fig. 1B). To obtain a higher efficiency for transfection, adenoviral particles expressing the probes were prepared using the ViraPower Adenoviral Expression System (Invitrogen, Carlsbad, CA, USA) following the manufacturer's instructions. The HEK293T cells were infected with the V2b-5C and VΔN-5C adenoviruses and lysed 48 h after the infection. In vitro emission spectra were described using an F-3000 Fluorescence Spectrophotometer (HITACHI, Tokyo, Japan) with excitation at 433 nm. The lysate from the V2b-5C-expressing cells grown in DMEM supplemented with 0.5% serum showed a peak at 533 nm, while 20% serum-containing medium abolished the effect (Fig. 1C). The emission ratio (Em_{475}/Em_{533}) of the serum-treated cells were calculated from the relative intensity of spectrum represented in Fig. 1C. When the average ratio in the low-serum-treated group was 1.00, the ratio from the high-serum-treated group was 1.15-fold higher than that of the low-serum-treated group (1.15±0.02; n=3, Fig. 1D). When mRNAs were depleted from lysate by RNase A, 533 nm-emission was recovered with an Em_{475}/Em_{533} ratio similar to the control (0.98±0.06; n=3, Fig. 1D). VΔN-5C-expressing lysate did not produce an emission peak at 533 nm in the presence or absence of serum stimulation (data not shown). These observations demonstrated that the serum-induced enhancement of the emission ratio was based on a specific interaction between the eIF moieties of the probes that are formed on the mRNA. To confirm whether protein synthesis was indeed activated by the serum, we performed a luciferase reporter assay treated with low or high concentration of serum under the