Distinct 3′UTRs differentially regulate activity-dependent translation of brain-derived neurotrophic factor (BDNF)

Anthony G. Lau[a], Hasan A. Irier[a], Jiaping Gu[b], Donghua Tian[a], Li Ku[a], Guanglu Liu[a], Mingjing Xia[a], Brita Fritsch[c], James Q. Zheng[b], Raymond Dingledine[a], Baoji Xu[d], Bai Lu[e], and Yue Feng[a,1]

Departments of [a]Pharmacology and [b]Cell Biology, Emory University School of Medicine, Atlanta, GA 30322; [c]Epilepsy Research Section, National Institute of Neurological Disorders and Stroke, National Institutes of Health, Bethesda, MD 20892; [d]Department of Pharmacology, Georgetown University School of Medicine, Washington, DC 20007; and [e]Section on Neural Development and Plasticity, National Institute of Child Health and Human Development, National Institutes of Health, Bethesda, MD 20892

Edited* by Dale Purves, Duke University Medical Center, Durham, NC, and approved July 15, 2010 (received for review March 9, 2010)

Expression of the brain-derived neurotrophic factor (BDNF) is under tight regulation to accommodate its intricate roles in controlling brain function. Transcription of BDNF initiates from multiple promoters in response to distinct stimulation cues. However, regardless which promoter is used, all BDNF transcripts are processed at two alternative polyadenylation sites, generating two pools of mRNAs that carry either a long or a short 3′UTR, both encoding the same BDNF protein. Whether and how the two distinct 3′UTRs may differentially regulate BDNF translation in response to neuronal activity changes is an intriguing and challenging question. We report here that the long BDNF 3′UTR is a bona fide *cis*-acting translation suppressor at rest whereas the short 3′UTR mediates active translation to maintain basal levels of BDNF protein production. Upon neuronal activation, the long BDNF 3′UTR, but not the short 3′UTR, imparts rapid and robust activation of translation from a reporter. Importantly, the endogenous long 3′UTR BDNF mRNA specifically undergoes markedly enhanced polyribosome association in the hippocampus in response to pilocarpine induced-seizure before transcriptional up-regulation of BDNF. Furthermore, BDNF protein level is quickly increased in the hippocampus upon seizure-induced neuronal activation, accompanied by a robust activation of the tropomyosin-related receptor tyrosine kinase B. These observations reveal a mechanism for activity-dependent control of BDNF translation and tropomyosin-related receptor tyrosine kinase B signaling in brain neurons.

alternative 3′UTR | tropomyosin-related kinase receptor B | hippocampal mossy fiber | epilepsy

Brain-derived neurotrophic factor (BDNF) is known to elicit a plethora of functions in the brain via activation of the tropomyosin-related receptor tyrosine kinase B (TrkB), ranging from neuronal survival and differentiation to circuit development and synaptic plasticity (1–3). Abnormalities in BDNF function have been implicated in both neurological and psychiatric disorders (4–6). To accommodate such diverse functions, a variety of mechanisms have evolved that tightly control BDNF expression. Transcription of the *BDNF* gene can be initiated from nine distinct promoters in mammals, allowing for sophisticated regulation by divergent extracellular and developmental cues (7–9). Moreover, the primary BDNF transcript can be processed at two alternative polyadenylation sites in all tissues examined, giving rise to two pools of BDNF mRNAs that harbor either a short or a long 3′UTR of 0.35 kb and 2.85 kb in length, respectively (8, 9). Each BDNF mRNA isoform encodes for the same BDNF protein. However, the relative abundance of the short and long 3′UTR BDNF mRNAs differ in various brain regions (10). The different 3′UTRs in BDNF mRNAs presumably interact with distinct *trans*-acting factors, thus offering a mechanism to increase the capacity and complexity for regulation of BDNF expression at posttranscriptional levels, such as translation and subcellular localization, which exceeds the traditional view of transcriptional regulation of BDNF.

In support of this idea, our previous study revealed distinct roles of the long and short 3′UTRs in controlling the localization of BDNF mRNA and the abundance of BDNF protein in neuronal soma and dendrites (10). The long 3′UTR, but not the short 3′UTR, is responsible for targeting BDNF mRNA into neuronal dendrites, which governs normal dendritic spine development and long-term potentiation (LTP) (10). These findings raise an intriguing question whether the long and short 3′UTRs may also differentially regulate BDNF translation in the somatal and dendritic compartments.

Given that activity-dependent transcription of BDNF is quite prominent, it is difficult to study whether neuronal activity can regulate BDNF translation. In this study, we show that the long and short 3′UTRs play differential roles in regulating BDNF translation in brain neurons. A majority of the hippocampal cytoplasmic BDNF mRNAs carry the short 3′UTR, which is actively translated and serves as the primary source of BDNF production at basal levels of neuronal activity. In contrast, the BDNF long 3′UTR is a bona fide suppressor for BDNF translation at rest. However, the long 3′UTR, but not the short 3′UTR, is sufficient to elicit rapid and activity-dependent translation from a reporter mRNA in cultured hippocampal neurons. Furthermore, upon seizure-induced neuronal activation, the endogenous long 3′UTR BDNF mRNA specifically undergoes robust translational activation in the hippocampus before transcriptional up-regulation of BDNF, which is temporally and spatially associated with a marked activation of TrkB. These results provide evidence for activity-dependent translational regulation of BDNF mediated by the distinct 3′UTRs, which offers a unique mechanism for controlling TrkB signaling to accommodate neuronal function.

Results

BDNF Long 3′UTR Is a *cis*-Acting Translation Suppressor at Rest. To directly test whether BDNF 3′UTRs may differentially influence translation, we constructed luciferase reporters fused with either the full-length BDNF 3′UTR (full), the long 3′UTR lacking the proximal polyadenylation site (long), or the short 3′UTR (short), which completely overlaps with the 5′ portion of the long 3′UTR (Fig. 1*A*). The parental luciferase construct that carries the bovine

NEUROSCIENCE

Author contributions: A.G.L. and Y.F. designed research; A.G.L., H.A.I., J.G., D.T., L.K., G.L., M.X., B.F., J.Q.Z., and Y.F. performed research; A.G.L., B.F., and J.Q.Z. analyzed data; and A.G.L., R.D., B.X., B.L., and Y.F. wrote the paper.

The authors declare no conflict of interest.

*This Direct Submission article had a prearranged editor.

[1]To whom correspondence should be addressed. E-mail: yfeng@emory.edu.

This article contains supporting information online at www.pnas.org/lookup/suppl/doi:10.1073/pnas.1002929107/-/DCSupplemental.

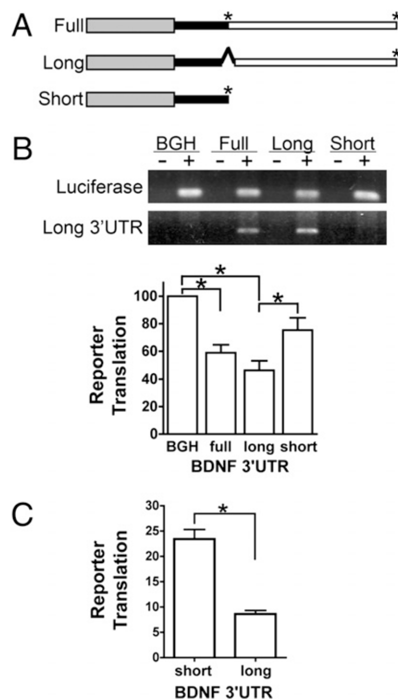

Fig. 1. The BDNF long 3′UTR is a bona fide *cis*-acting translation suppressor. (*A*) Schematic of luciferase reporter constructs harboring full-length BDNF 3′UTR, short BDNF 3′UTR, or a deletion mutant that produces only BDNF long 3′UTR. Asterisks indicate polyadenylation sites. (*B*) BDNF long 3′UTR suppresses reporter translation in transfected CAD cells, an immortalized neuronal cell line derived from mouse brain. *Top*: RT-PCR demonstrates the expression of luciferase reporter mRNAs that carry the BGH 3′UTR, the BDNF full, long, or short 3′UTRs using primers indicated on the left. − and + indicate the absence and presence of reverse transcriptase. *Bottom*: Luciferase activity normalized to qRT-PCR reading of corresponding reporter mRNAs (one-way ANOVA, $P < 0.05$, $n = 4$; *$P < 0.05$, Tukey analysis). Error bars indicate SEM. (*C*) The long and short BDNF 3′UTRs differentially mediate luciferase translation when expressed in primary cultured hippocampal neurons (DIV3). Significantly lower luciferase activity was detected from cells transfected with the BDNF long 3′UTR construct compared with that from the BDNF short 3′UTR construct (*$P < 0.05$, Student t test, $n = 6$).

growth hormone (BGH) 3′UTR was used as a high level expression control. When expressed in an immortalized brain neuron cell line, translation of luciferase reporter mRNAs carrying the short BDNF 3′UTR or the BGH 3′UTR were highly active (Fig. 1*B*). In contrast, the long BDNF 3′UTR reporter displayed significantly lower translation activity ($P < 0.05$). The BDNF full-length 3′UTR that can be processed at both polyadenylation sites mediated an intermediate level of reporter translation (Fig. 1*B*), further supporting the conclusion that the long 3′UTR suppresses translation. The differential translatability mediated by the inherent *cis*-acting properties in the short and long BDNF 3′UTRs was also observed in cultured hippocampal neurons (Fig. 1*C*).

Because all BDNF mRNA isoforms encode the same BDNF protein, the influence of individual 3′UTRs on BDNF translation in vivo cannot be assessed by simply measuring changes in BDNF protein expression. Therefore, we performed a linear sucrose gradient fractionation assay, which is commonly used to evaluate translation efficiency of an mRNA by measuring its ability to carry translating polyribosomes (11, 12). Sedimentation of ribosome-free ribonucleoprotein particles (mRNPs; fraction 1–3), the 80S monoribosome (fraction 3), and polyribosomes (fraction 4–10) in mouse hippocampal lysates were monitored by absorption at the wavelength of 254 nm (Fig. 2*A Top*). Quantitative RT-PCR (qRT-PCR) analysis showed that the BDNF long 3′UTR mRNA was largely sequestered into complexes not engaged with translating polyribosomes (fraction 1–3; Fig. 2*A Middle*), similar to sedimentation of EDTA-released ribosome-free mRNPs (Fig. 2*B*). In contrast, pan-BDNF mRNA and GAPDH mRNA were predominantly associated with polyribosomes (fraction 4–10; Fig. 2*A*). The BDNF short 3′UTR completely overlaps with the 5′ portion of the long 3′UTR and thus cannot be directly quantified by RT-PCR in the gradient fractions. However, in hippocampal cytoplasmic extracts, at least 75% of pan-BDNF mRNAs carry the short 3′UTR (Fig. S1). Thereby, the predominant polyribosome association of the pan-BDNF mRNA is largely mediated by the short 3′UTR. Semiquantitative RT-PCR allowed visualization of the aforementioned mRNA species in the gradient fractions (Fig. 2 *A* and *B Bottom*), confirming their translation status. The identification of BDNF long 3′UTR mRNA in dormant mRNPs (fraction 2) extends the previous observation regarding low abundance of this mRNA in crude polyribosome pellet (13), suggesting that the long 3′UTR suppresses BDNF translation at the step of initiation. Together, these data suggest that, whereas the short 3′UTR mediates active translation of BDNF at rest, the long 3′UTR is a bona fide *cis*-acting translation suppressor in vitro and in vivo.

BDNF Long 3′UTR Specifically Mediates Activity-Dependent Translation. To address whether and how BDNF 3′UTRs regulate translation upon neuronal activation, destabilized EGFP reporters (d2EGFP) carrying the long or short BDNF 3′UTR or the SV40 3′UTR were transfected individually into cultured hippocampal neurons [16 days in vivo (DIV); Fig. 3]. The d2EGFP reporter harbors a membrane insertion tag for limiting diffusion of newly synthesized d2EGFP from the site of translation (14). After the synaptic network was well established (at DIV 21), the culture was treated with tetraethylammonium (TEA) using conditions known to induce chemical LTP (15). Neuronal activation was evidenced by a drastic up-regulation of c-fos mRNA 10 min after TEA exposure (Fig. S2). Live cell laser confocal imaging demonstrated that TEA treatment elicited a rapid increase of d2EGFP expression in a BDNF long 3′UTR–dependent manner, which is clearly visualized in soma and dendrites (Fig. 3 *E* and *F*). Quantitative analysis further demonstrated that d2EGFP was indeed increased in both dendrites and soma (Fig. 3 *G* and *H*), suggesting that the BDNF long 3′UTR is sufficient for mediating activity-dependent translation in the somatal and dendritc compartments. In contrast, neither the short BDNF 3′UTR nor the SV40 3′UTR could mediate activity-dependent d2EGFP expression (Fig. 3 *A–D*, *G*, and *H*).

To address the key question whether and how neuronal activity regulates BDNF translation in vivo, we analyzed polyribosome association of hippocampal BDNF mRNAs in pilocarpine- and vehicle-treated rats. A single pilocarpine administration reliably induced stage V seizures, followed by increased seizure intensity, duration, and frequency, designated as status epilepticus (SE) (16). The onset of stage V seizure was used to mark the starting point of SE. Both the pan and the long 3′UTR BDNF mRNAs were markedly up-regulated 90 min after pilocarpine-induced SE and returned to basal levels 24 h later (Fig. 4*A*). However, only a slight increase of pan-BDNF mRNA was detected 30 min after SE induction, whereas no detectable changes were observed for the long 3′UTR BDNF mRNA (Fig. 4*A*). Importantly, before transcriptional up-regulation of BDNF, a robust translational activation of the long 3′UTR BDNF mRNA was observed 30 min after SE induction, indicated by the marked shift of the long 3′UTR BDNF mRNA from translationally inactive mRNPs to polyribosome-associated complexes (Fig. 4*B*). The activity-dependent engagement of the long 3′UTR BDNF mRNA into active translation elongation was further evidenced by the release of this mRNA into ribosome-free mRNPs upon puromycin treatment (Fig. S3), which specifically dissociates actively translating polyribosomes (17). Conversely, pan-BDNF mRNA, in which the majority harbors the short 3′UTR (Fig. S1), was se-

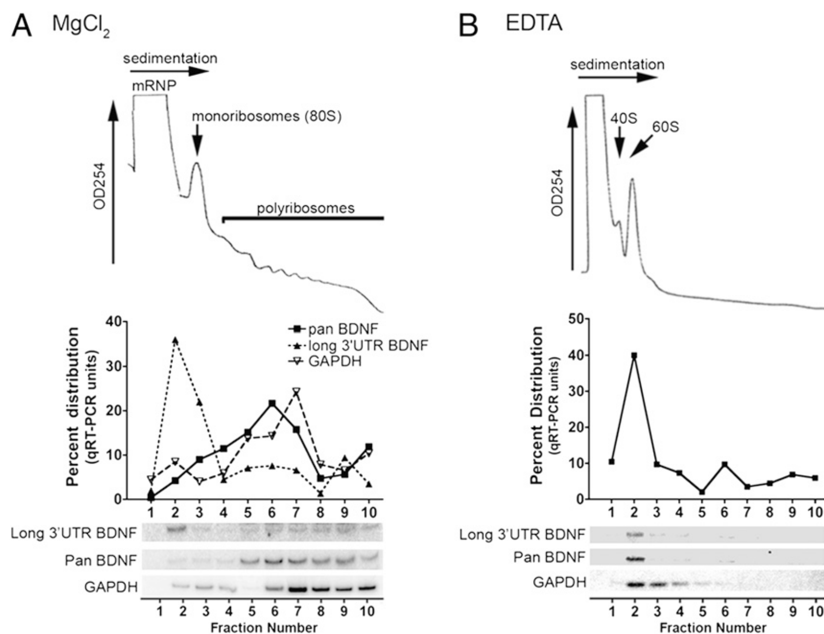

Fig. 2. Endogenous long 3′UTR BDNF mRNA in mouse hippocampus was translationally repressed. (*A*) Cytoplasmic extracts from mouse hippocampi were prepared in the presence of $MgCl_2$ for linear sucrose gradient fractionation (15–45%) to separate translating polyribosomes (fraction 4–10) from nontranslating components including dormant mRNPs, ribosome subunits, and monoribosomes (fraction 1–3), monitored by absorption at OD254 (*Top*). qRT-PCR was performed to determine the distribution of the pan-BDNF mRNA, the long 3′UTR-BDNF mRNA, and the housekeeping GAPDH mRNA in each gradient fraction (*Middle*). Phosphorus 32–labeled semiquantitative RT-PCR products of the aforementioned mRNAs were visualized by a PhosphorImager (*Bottom*). (*B*) Hippocampal lysates were treated with EDTA to release mRNAs from polyribosomes to ribosome-free mRNPs. Note the disappearance of mono- and polyribosomes, and the accumulation of released 40S and 60S ribosome subunits on the linear sucrose gradient (*Top*). qRT-PCR for the long 3′UTR BDNF mRNA (*Middle*) and ^{32}P-labeled semiquantitative RT-PCR products (*Bottom*) for the long 3′UTR BDNF mRNA, the pan BDNF mRNA, and GAPDH mRNA were indicated respectively.

questered into ribosome-free mRNPs upon SE (Fig. 4*C*). Similar effects on the changes of polyribosome association by the long and pan-BDNF mRNAs were observed in mice in response to pilocarpine-induced SE (Fig. S4). In contrast to the dramatic translational enhancement of BDNF mediated by the long 3′UTR, BDNF mRNAs containing 5′UTRs encoded by exons IV and VI, which are most abundant in adult hippocampus (Fig. 4*D*) and known to be up-regulated by neuronal activation (18), displayed slight reduction of polyribosome association (Fig. 4 *E* and *F*). Thus, these 5′UTRs are not responsible for the activity-dependent translation of BDNF (Fig. 4*B*).

Activity-Dependent Translation of BDNF Mediated by the Long 3′UTR Leads to Robust TrkB Activation in Hippocampal Mossy Fibers. The long 3′UTR BDNF mRNA is abundant in the dentate gyrus (DG) and CA3 (Fig. S5) and subjected to activity-stimulated polyribosome association (Fig. 4*B*). The BDNF protein synthesized in DG is transported and stored at high concentrations in the hippocampal mossy fiber (MF) axonal terminals that form synapses with CA3 pyramidal neurons (19). Seizure-induced neuronal activation releases BDNF from the MF synapses, which leads to robust activation of TrkB in the MF tract (19, 20). Accompanied with the activity-stimulated polyribosome association of the BDNF long 3′UTR mRNA (Fig. 4*B*), BDNF protein was significantly increased within and near the MF tract, with the highest level in CA3 strata lucidum and radiatum (Fig. 5*A*). Importantly, polyribosome association by the pan-BDNF mRNA was reduced (Fig. 4*C*) and the BDNF long 3′UTR mRNA expression was not up-regulated at this time point (Fig. 4*A*). Thus, the increase of BDNF protein is a result of long 3′UTR-mediated translation enhancement upon neuronal activation. Furthermore, the increase of BDNF protein is temporally and spatially associated with TrkB activation in the MF tract, indicated by phosphorylation of tyrosine 816 (pY816) of TrkB (Fig. 5*B*). Together, these results suggest that activity-dependent BDNF translation contributes to TrkB activation in vivo, and hippocampal MF circuitry is apparently a primary site for such regulation during pilocarpine-induced seizure.

Discussion

Our results provide evidence that the long and short 3′UTRs differentially govern BDNF translation to accommodate neuronal activity changes, independent of transcriptional up-regulation of BDNF. Under resting conditions, the short 3′UTR restricts BDNF mRNA to the neuronal soma, which is clearly seen in cultured hippocampal neurons (10). We show here that the short BDNF mRNA is predominantly associated with translating polyribosomes in the hippocampus, which maintains basal levels of BDNF production. In contrast, the long 3′UTR BDNF mRNA, which is enriched in dendrites in culture (10), is largely sequestered into translationally dormant mRNPs. Upon neuronal activation, the BDNF long 3′UTR, but not the short 3′UTR, is sufficient to mediate activity-dependent translation of reporter in both the somatal and dendritic compartments. These results raise an intriguing possibility that, under physiological stimuli, the long 3′UTR-mediated BDNF translation may be elicited in selectively activated dendrites and synapses, which in turn can be released to strengthen the nearby synapses. Our previous finding that LTP in CA1 apical dendrites was selectively impaired in the absence of the BDNF long 3′UTR (10) is consistent with this idea. The recent finding that synaptic activity-stimulated dendritic BDNF synthesis by eEF2-signaling modulates dendritic spine morphology (21) further supports this hypothesis. On the contrary, upon robust neuronal activation in pilocarpine-induced seizure, activity-dependent BDNF translation mediated by the long 3′UTR is most likely evoked in the entire neuron, not limited to dendrites. Such regulation apparently serves as the primary source for BDNF production immediately after seizure, because translation of the short 3′UTR BDNF mRNA is suppressed under such a con-

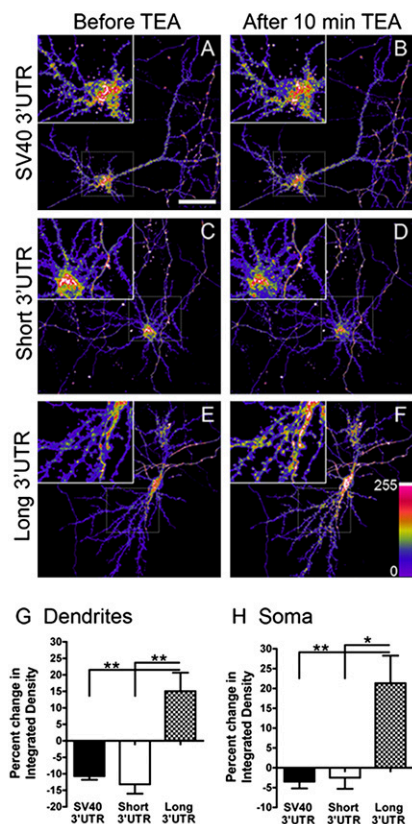

Fig. 3. BDNF long 3′UTR sufficiently mediates activity-stimulated reporter translation. Laser confocal live cell imaging of cultured hippocampal neurons (DIV21) expressing d2EGFP fused with the SV40 3′UTR (*A* and *B*) or the short (*C* and *D*) or the long BDNF 3′UTR (*E* and *F*) before and after 10 min TEA exposure. (Scale bar: 40 μm.) Dotted boxes are magnified (2×) and shown as insets. The pseudocolor bar indicates fluorescent intensities. (*G* and *H*) Quantification of the changes in d2EGFP reporter fluorescence caused by TEA. A randomly selected neuron was imaged from each coverslip by z-sectioning through the entire cell (10–15 optical slices) and projected into a 2D image (maximum intensity) before and after TEA exposure. The total integrated fluorescent density in dendrites (*G*) and soma (*H*) of the same cell before and after 10 min TEA exposure was measured. The percent change of fluorescence was calculated by normalizing the integrated intensity after TEA treatment against that of the same cell before TEA treatment, and results were displayed graphically. The decrease of fluorescence for reporters that carry the BDNF short 3′UTR and the SV40 3′UTR may reflect photobleaching during confocal imaging. One-way ANOVA analysis: $P < 0.05$ for both soma and dendrites ($n = 4$). Error bars indicate SEM. $^{**}P < 0.01$, Tukey analysis.

dition. The rapid and robust translational activation of BDNF via the long 3′UTR before increased transcription of BDNF provides a unique means to modulate TrkB activation, which may have important implications in normal and pathological plasticity, considering the critical roles of TrkB signaling in cognitive function and epileptogenesis (4, 22, 23).

Most mammalian genes express alternative UTRs (24). Our studies provide an example that alternative 3′UTRs can elicit opposing effects on translation to regulate expression of the same encoded protein in response to neuronal activity changes. The reciprocal shift between dormant mRNPs and polyribosomes of BDNF mRNAs harboring the long or short 3′UTRs upon pilocarpine-induced seizure suggests that activity-dependent regulation is at the step of translation initiation. Such regulation is most likely mediated by unidentified *trans*-acting factors including RNA-binding proteins and microRNAs that differentially bind the distinct BDNF 3′UTRs. Interestingly, mRNAs are transported to dendrites as translationally repressed granules (25, 26), where synaptic stimulation results in local translational activation (27). Whether translational suppression of BDNF by the long 3′ UTR may facilitate dendritic localization of the long 3′UTR BDNF mRNA is an intriguing possibility to be explored. In addition, whether the same or distinct *cis*- and *trans*-acting factors are responsible for dendritic localization and translation regulation of BDNF are the next challenges.

Besides the alternative 3′UTRs, BDNF mRNAs harbor nine distinct 5′UTRs (7, 8), among which exons II and VI are also thought to direct dendritic localization of the BDNF mRNA (18). All of the alternatively expressed 5′UTRs are linked with either the short or the long BDNF mRNA (13, 28). However, these 5′UTRs are unlikely responsible for the activity evoked translation of BDNF upon pilocarpine-induced seizure. This is consistent with our observation that the BDNF long 3′UTR is sufficient to mediate synaptic activity-dependent translation of reporter in the TEA stimulation paradigm without BDNF 5′ UTRs. However, whether and how the 5′UTRs may facilitate the long 3′UTR–dependent translation regulation and/or dendritic localization, especially after the robust transcriptional up-regulation of BDNF in later stages of seizure (29), remains to be elucidated by future studies.

BDNF is the primary ligand for TrkB activation in the brain, which is most enriched in the axonal terminals of the hippocampal MFs (19). Although TrkB activation can be achieved by other non–neurotrophin-based mechanisms, such as Zinc-mediated and Src-dependent transactivation of TrkB (30), the potency for BDNF to activate TrkB is higher by hundreds of fold (30). The abundant expression of the long 3′UTR BDNF mRNA in DG and CA3 suggests that these neurons are the primary sites for activity-dependent BDNF translation in the hippocampus. BDNF proteins that are made in the soma of DG granule cells (DGCs) and transported into MF terminals, and BDNF proteins that are synthesized in the somatal and dendritic compartments of CA3 neurons, can be secreted at the MF synapses to activate TrkB in the hippocampal MF tract. These results support the previous finding that hippocampal MFs represent the primary circuit for BDNF-TrkB signaling in epileptogenesis (19). It is critical to point out that exposure to a rapid surge of BDNF only evokes a transient TrkB activation that diminishes quickly within minutes (31, 32). In contrast, prolonged TrkB activation requires a progressive increase in BDNF concentration (32). Thus, the marked TrkB activation in MFs after sustained seizure in pilocarpine-induced SE is unlikely achieved solely by secretion of stored BDNF. Instead, increased release of newly synthesized BDNF must be involved. Importantly, the robust TrkB activation is achieved when translation of the pan BDNF mRNA is largely repressed. Hence, activity-dependent translation of BDNF from the long 3′UTR mRNA, likely in the presynaptic DGCs or the postsynaptic CA3 neurons, or both, is the primary source for TrkB activation in the MFs.

In conclusion, the distinct BDNF 3′UTRs differentially regulate BDNF translation in the somatal and dendritic compartments, providing a unique means to precisely control the temporal and spatial production of BDNF and TrkB signaling in response to neuronal activation. Such a regulatory mechanism could be employed by the growing numbers of neuronal mRNAs identified that harbor alternative 3′UTRs, thus increasing the complexity for regulating expression of the same encoded protein to accommodate sophisticated neuronal function in response to various extracellular stimuli.

Materials and Methods

Constructs. The firefly luciferase coding sequence from the pGL3-basic vector (Promega) was inserted into pcDNA3 (Invitrogen). The short 3′UTR, the full-length 3′UTR, and the long 3′UTR lacking the proximal polyadenylation site were inserted downstream of the luciferase coding region individually. Each of the aforementioned 3′UTRs was also subcloned into the d2EGFP vector (14).

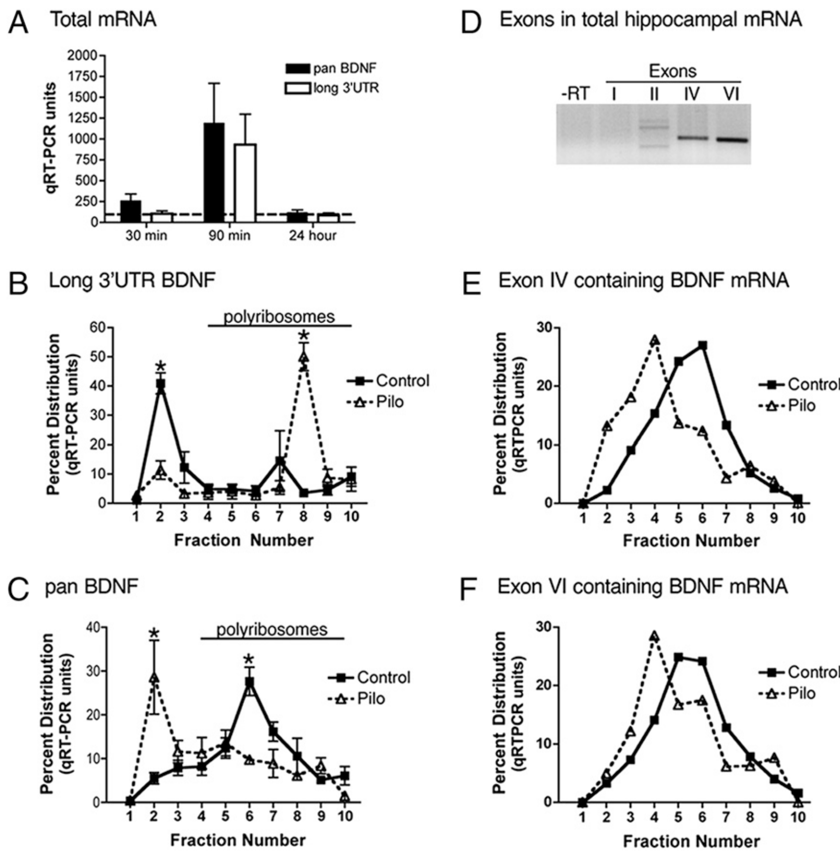

Fig. 4. Pilocarpine-induced neuronal activation results in robust translational derepression of BDNF specifically mediated by the long 3′UTR. (*A*) Levels of pan-BDNF mRNA and long 3′UTR BDNF mRNA by qRT-PCR in rat hippocampus after pilocarpine-induced SE at the indicated time points. Basal levels of corresponding BDNF mRNAs normalized to that of the GAPDH mRNA were set at 100% (dotted line). qRT-PCR of long 3′UTR BDNF mRNA (*B*) and pan-BDNF mRNA (*C*) in sucrose gradient fractions of hippocampal extracts from control (■) or pilocarpine (Pilo)–treated rats (△) after 30 min of SE. $P < 0.05$, two-way ANOVA; $*P < 0.05$ Bonferroni posttest. (*D*) Semiquantitative RT-PCR of the 5′ UTRs in BDNF mRNA indicates that exon IV and VI are the major 5′UTRs expressed in the adult rat hippocampus. Polyribosomal profiles of BDNF mRNAs carrying exon IV (*E*) and exon VI (*F*) determined by linear sucrose gradient fractionation qRT-PCR assay using hippocampal lysates from control or Pilo-treated rats after 30 min of SE. Error bars indicate SEM.

Cells, Transfection, and Luciferase Assay. CAD cells were raised as previously described (33). Each firefly luciferase reporter construct was cotransfected with the pTK-Renilla plasmid (Promega) using Lipofectamine 2000 (Invitrogen). Twenty-four hours after transfection, dual luciferase assay was performed (Promega). Total RNA was isolated from an aliquot of cells from each transfection, followed by regular RT-PCR and qRT-PCR as indicated. The levels of each reporter mRNA were determined by normalization of the qRT-PCR reading by firefly luciferase coding region primers to that of GAPDH. Translation activity from each construct was estimated by normalizing firefly luciferase readings to firefly luciferase mRNA levels determined by qRT-PCR.

Primary hippocampal neurons were raised from embryonic day 18 Sprague-Dawley rats (34), and cotransfected at DIV3 using Lipofectamine 2000 (Invitrogen) with the firefly luciferase reporter along with the Renilla luciferase plasmid. Cell lysates were harvested 24 h after transfection and subjected to dual luciferase assay (Promega).

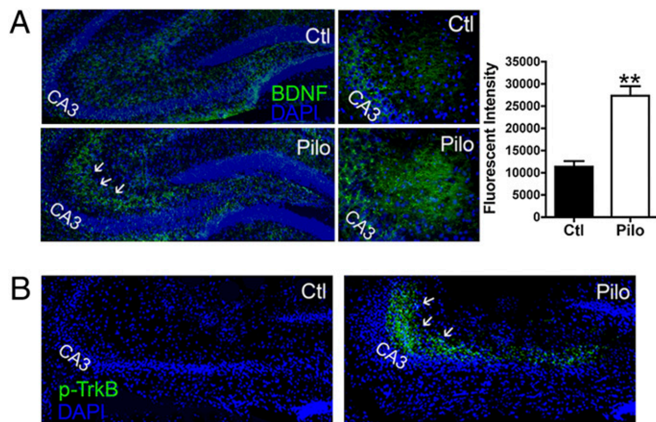

Fig. 5. Increased BDNF protein and TrkB activation upon seizure-induced neuronal activation in the hippocampus associated with activity-dependent translation of the long 3′UTR BDNF mRNA. (*A*) Immunofluorescence of BDNF (green) is increased in hippocampal MF tract 30 min after pilocarpine-induced SE (arrows, *Left Lower*). The increase of BDNF signal is more clearly observed in the CA3 strata lucidum and radiatum under higher magnification (*Center*), which is quantified and graphically displayed (*Right*). Error bars indicate SEM; $**P < 0.01$ Student *t* test ($n = 3$). (*B*) pY816-TrkB immunofluorescence (green) is drastically increased in hippocampal MFs 30 min after pilocarpine-induced SE (arrows, *Right*). For all images, the nuclear staining by DAPI (blue) marks the principle neuron layers.

Pilocarpine-Induced Seizure. Animals were treated in accordance with National Institutes of Health regulations under the approval of the Emory University Institutional Animal Care and Use Committee. Pilocarpine reliably induced stage V seizures in adult male Sprague-Dawley rats and C57 Bl/6 mice (Charles River Labs), characterized by distinct motor behaviors including forelimb clonus, loss of postural control, rearing, and falling (35). Animals presenting these behaviors with increased seizure intensity, duration and frequency 20 to 40 min after the injection of pilocarpine were declared to be in SE, and the initial onset of stage V seizure was used to mark the start point of SE.

Linear Sucrose Gradient Fractionation. Hippocampi were homogenized in a gradient buffer (20 mM Tris, pH 7.5, 100 mM KCl, 5 mM $MgCl_2$) containing 100 μg/mL cycloheximide to arrest polyribosome migration and then lysed with 1% Triton X-100. Cytoplasmic extracts were fractionated on 15% to 45% (wt/vol) sucrose gradient and total RNA was extracted as previously described (12, 35). To dissociate polyribosomes, EDTA-treated lysate was centrifuged on a parallel gradient containing 1 mM EDTA. Puromycin treatment was carried out as described in a previous report (17).

TEA-Evoked Neuronal Activation. Primary hippocampal neurons from embryonic day 18 Sprague-Dawley rats cultured on coverslips (34) were transfected using CalPhos mammalian transfection kit (Clontech) with d2EGFP reporter constructs on DIV16 and subjected to live imaging on DIV21. Before imaging, culture medium was replaced by Hepes-buffered recording solution (140 mM NaCl, 5 mM KCl, 2 mM $CaCl_2$, 1.5 mM $MgCl_2$, 10 mM glucose, 25 mM Hepes, pH 7.4), and coverslips were mounted onto a heating chamber maintained at 37 °C.

For each coverslip, only one randomly selected single neuron expressing the reporter was imaged using Nikon C1 laser scanning confocal system on a Nikon inverted microscope. Confocal z-sectioning through the entire cell (10–15 optical slices) was performed to obtain a 2D image with maximal intensity projection. The first image was taken while neurons were incubated in Hepes-buffered recording solution. The second image was taken from the same cell 10 min after incubation with TEA in chemical LTP medium (140 mM NaCl, 5 mM KCl, 5 mM $CaCl_2$, 0.1 mM $MgCl_2$, 10 mM glucose, 25 mM Hepes, pH 7.4, and 25 mM TEA). To reliably detect any changes in fluorescence, each cell was imaged before and after TEA treatment with the exact same parameters (laser power, pinhole size, gain, contrast, and section number). All the images were background-subtracted before analysis. The integrated intensities were quantitatively analyzed using ImageJ software (National Institutes of Health). The intensity of the pyramidal cell soma was quantified by enclosing it in a polygon box. The total dendritic signals from the same cell were derived by subtracting the soma intensity from that of the entire neuron. Almost all processes imaged were dendritic, characterized by numerous spines, with only few thin axonal segments showing weak fluorescence. Finally, percent changes of intensity caused by TEA treatment in the soma and dendrites of each neuron were calculated by normalization against the intensity in the corresponding subcellular compartments of the same neuron before TEA treatment.

Immunocytochemistry. For BDNF staining, brain slices were blocked in PBS solution containing 0.01% Triton X-100, 2% BSA (Sigma), and 2% normal goat serum for 1 h before being incubated overnight at 4 °C with anti-BDNF antibody (1:2,000; Santa Cruz Biotechnology). Fluorescent signals were detected using a Olympus IX-51 microscope equipped with a Retica digital camera and quantified using ImageJ (National Institutes of Health). For pTrkB staining, brain slices were blocked in PBS solution containing 0.01% Triton X-100, 2% BSA and 1 mM sodium orthovanadate (Sigma) for 1 h before being incubated overnight at 4 °C with pY816-TrkB antibody (36) (1:200, provided by Moses Chao, Skirball Institute, New York University, New York, NY). After washing, slices were incubated with anti–rabbit FITC (1:1,000; Jackson Immunoresearch) at room temperature for 1 h. Fluorescent signals were detected by using a Zeiss LSM 510 confocal microscopic imaging system.

RT-PCR and Real-Time RT-PCR. Total RNA was reverse transcribed using random primers (Promega) and SuperScript II RNaseH-reverse transcriptase (Invitrogen), followed by PCR analysis. qPCR was performed using iCYCLER (BioRad) and SYBR-green master mix (NEB). The following primer pairs were used: pan BDNF, 5′-gccgcaaacatgtctatgagggtt-3′ (forward), 5′-ttggcctttggataccgggacttt-3′ (reverse); long3′UTR BDNF, 5′-caggaggaatttctgagtggcca-3′ (forward), 5′-gcagaaggcctaagcaacttgaca-3′ (reverse); firefly luciferase, 5′-aagattcaaagtgcgctgctggtg-3′ (forward), 5′-ccgcttccccgacttccttagag-3′ (reverse); and cfos, 5′-agaaggggcaaagtagagcag-3′ (forward), 5′-cgcagacttctcgtcttcaagt-3′ (reverse). Previously published primers were used to detect BDNF mRNAs that carry various 5′ exons (7) and GAPDH mRNA (37).

Statistical Analysis. The Student *t* test was used for two-sample comparisons. For multiple sample comparisons, one-way ANOVA was performed followed by Tukey posttest or two-way ANOVA analysis followed by Bonferroni posttest.

ACKNOWLEDGMENTS. We thank Geidy Serrano (Emory University, Atlanta, GA) for her assistance with primary hippocampal neuron cultures and pilocapine-induced SE in mice. The anti–pY816-TrkB antibody was a gift from Dr. Moses Chao (Skirball Institute at New York University, New York, NY). The d2EGFP reporter was provided by Dr. Gary Bassell (Emory University, Atlanta, GA). This study was supported by National Institutes of Health Grants NS056532, 5T32GM008602 (to A.L.), HD061344 (to Y.F.), NS050596 (to B.X.), and NS036604 (to R.D.).

1. Zhou Z, et al. (2006) Brain-specific phosphorylation of MeCP2 regulates activity-dependent Bdnf transcription, dendritic growth, and spine maturation. *Neuron* 52: 255–269.
2. Lu Y, Christian K, Lu B (2008) BDNF: A key regulator for protein synthesis-dependent LTP and long-term memory? *Neurobiol Learn Mem* 89:312–323.
3. Yoshii A, Constantine-Paton M (2010) Postsynaptic BDNF-TrkB signaling in synapse maturation, plasticity, and disease. *Dev Neurobiol* 70:304–322.
4. Martinowich K, Manji H, Lu B (2007) New insights into BDNF function in depression and anxiety. *Nat Neurosci* 10:1089–1093.
5. Binder DK, Croll SD, Gall CM, Scharfman HE (2001) BDNF and epilepsy: Too much of a good thing? *Trends Neurosci* 24:47–53.
6. Buckley PF, Mahadik S, Pillai A, Terry A, Jr (2007) Neurotrophins and schizophrenia. *Schizophr Res* 94:1–11.
7. Aid T, Kazantseva A, Piirsoo M, Palm K, Timmusk T (2007) Mouse and rat BDNF gene structure and expression revisited. *J Neurosci Res* 85:525–535.
8. Liu QR, et al. (2006) Rodent BDNF genes, novel promoters, novel splice variants, and regulation by cocaine. *Brain Res* 1067:1–12.
9. Liu QR, et al. (2005) Human brain derived neurotrophic factor (BDNF) genes, splicing patterns, and assessments of associations with substance abuse and Parkinson's disease. *Am J Med Genet B Neuropsychiatr Genet* 134B:93–103.
10. An JJ, et al. (2008) Distinct role of long 3′ UTR BDNF mRNA in spine morphology and synaptic plasticity in hippocampal neurons. *Cell* 134:175–187.
11. Zalfa F, Achsel T, Bagni C (2006) mRNPs, polysomes or granules: FMRP in neuronal protein synthesis. *Curr Opin Neurobiol* 16:265–269.
12. Lu R, et al. (2004) The fragile X protein controls microtubule-associated protein 1B translation and microtubule stability in brain neuron development. *Proc Natl Acad Sci USA* 101:15201–15206.
13. Timmusk T, Persson H, Metsis M (1994) Analysis of transcriptional initiation and translatability of brain-derived neurotrophic factor mRNAs in the rat brain. *Neurosci Lett* 177:27–31.
14. Aakalu G, Smith WB, Nguyen N, Jiang C, Schuman EM (2001) Dynamic visualization of local protein synthesis in hippocampal neurons. *Neuron* 30:489–502.
15. Aniksztejn L, Ben-Ari Y (1991) Novel form of long-term potentiation produced by a K+ channel blocker in the hippocampus. *Nature* 349:67–69.
16. Racine RJ (1972) Modification of seizure activity by electrical stimulation. II. Motor seizure. *Electroencephalogr Clin Neurophysiol* 32:281–294.
17. Stefani G, Fraser CE, Darnell JC, Darnell RB (2004) Fragile X mental retardation protein is associated with translating polyribosomes in neuronal cells. *J Neurosci* 24: 7272–7276.
18. Chiaruttini C, Sonego M, Baj G, Simonato M, Tongiorgi E (2008) BDNF mRNA splice variants display activity-dependent targeting to distinct hippocampal laminae. *Mol Cell Neurosci* 37:11–19.
19. Danzer SC, McNamara JO (2004) Localization of brain-derived neurotrophic factor to distinct terminals of mossy fiber axons implies regulation of both excitation and feedforward inhibition of CA3 pyramidal cells. *J Neurosci* 24:11346–11355.
20. He XP, Pan E, Sciarretta C, Minichiello L, McNamara JO (2010) Disruption of TrkB-mediated phospholipase Cgamma signaling inhibits limbic epileptogenesis. *J Neurosci* 30:6188–6196.
21. Verpelli C, et al. (2010) Synaptic activity controls dendritic spine morphology by modulating eEF2-dependent BDNF synthesis. *J Neurosci* 30:5830–5842.
22. Yoshii A, Constantine-Paton M (2010) Postsynaptic BDNF-TrkB signaling in synapse maturation, plasticity, and disease. *Dev Neurobiol* 70:304–322.
23. He XP, et al. (2004) Conditional deletion of TrkB but not BDNF prevents epileptogenesis in the kindling model. *Neuron* 43:31–42.
24. Hughes TA (2006) Regulation of gene expression by alternative untranslated regions. *Trends Genet* 22:119–122.
25. Huang YS, Jung MY, Sarkissian M, Richter JD (2002) N-methyl-D-aspartate receptor signaling results in Aurora kinase-catalyzed CPEB phosphorylation and alpha CaMKII mRNA polyadenylation at synapses. *EMBO J* 21:2139–2148.
26. Hüttelmaier S, et al. (2005) Spatial regulation of beta-actin translation by Src-dependent phosphorylation of ZBP1. *Nature* 438:512–515.
27. Bassell GJ, Kelic S (2004) Binding proteins for mRNA localization and local translation, and their dysfunction in genetic neurological disease. *Curr Opin Neurobiol* 14: 574–581.
28. Timmusk T, et al. (1993) Multiple promoters direct tissue-specific expression of the rat BDNF gene. *Neuron* 10:475–489.
29. Tongiorgi E, et al. (2004) Brain-derived neurotrophic factor mRNA and protein are targeted to discrete dendritic laminas by events that trigger epileptogenesis. *J Neurosci* 24:6842–6852.
30. Huang YZ, Pan E, Xiong ZQ, McNamara JO (2008) Zinc-mediated transactivation of TrkB potentiates the hippocampal mossy fiber-CA3 pyramid synapse. *Neuron* 57: 546–558.
31. Soppet D, et al. (1991) The neurotrophic factors brain-derived neurotrophic factor and neurotrophin-3 are ligands for the trkB tyrosine kinase receptor. *Cell* 65:895–903.
32. Ji Y, et al. (2010) Acute and gradual increases in BDNF concentration elicit distinct signaling and functions in neurons. *Nat Neurosci* 13:302–309.
33. Wang H, et al. (2008) Dynamic association of the fragile X mental retardation protein as a messenger ribonucleoprotein between microtubules and polyribosomes. *Mol Biol Cell* 19:105–114.
34. Banker GA, Cowan WM (1977) Rat hippocampal neurons in dispersed cell culture. *Brain Res* 126:397–42.
35. Irier HA, Shaw R, Lau A, Feng Y, Dingledine R (2009) Translational regulation of GluR2 mRNAs in rat hippocampus by alternative 3′ untranslated regions. *J Neurochem* 109: 584–594.
36. Bath KG, et al. (2008) Variant brain-derived neurotrophic factor (Val66Met) alters adult olfactory bulb neurogenesis and spontaneous olfactory discrimination. *J Neurosci* 28:2383–2393.
37. Zhao L, Tian D, Xia M, Macklin WB, Feng Y (2006) Rescuing qkV dysmyelination by a single isoform of the selective RNA-binding protein QKI. *J Neurosci* 26:11278–11286.