Localized IRES-Dependent Translation of ER Chaperone Protein mRNA in Sensory Axons

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Abstract

Transport of neuronal mRNAs into distal nerve terminals and growth cones allows axonal processes to generate proteins autonomous from the cell body. While the mechanisms for targeting mRNAs for transport into axons has received much attention, how specificity is provided to the localized translational apparatus remains largely unknown. In other cellular systems, protein synthesis can be regulated by both cap-dependent and cap-independent mechanisms. The possibility that these mechanisms are used by axons has not been tested. Here, we have used expression constructs encoding axonally targeted bicistronic reporter mRNAs to determine if sensory axons can translate mRNAs through cap-independent mechanisms. Our data show that the well-defined IRES element of encephalomyocarditis virus (EMCV) can drive internal translational initiation of a bicistronic reporter mRNA in distal DRG axons. To test the potential for cap-independent translation of cellular mRNAs, we asked if calreticulin or grp78/BiP mRNA 5'UTRs might have IRES activity in axons. Only grp78/BiP mRNA 5'UTR showed clear IRES activity in axons when placed between the open reading frames of diffusion limited fluorescent reporters. Indeed, calreticulin's 5'UTR provided an excellent control for potential read through by ribosomes, since there was no evidence of internal initiation when this UTR was placed between reporter ORFs in a bicistronic mRNA. This study shows that axons have the capacity to translate through internal ribosome entry sites, but a simple binary choice between cap-dependent and cap-independent translation cannot explain the specificity for translation of individual mRNAs in distal axons.

Introduction

Eukaryotic cells can temporally and spatially regulate protein composition of subcellular domains through translation of mRNAs transported to these sites. This is particularly relevant to neurons where both the post-synaptic and pre-synaptic processes can be separated from the cell body by long distances. Initial studies suggested that localized protein synthesis in neurons is restricted to dendrites. However, several different laboratories have demonstrated that axons contain ribosomes, translation factors and mRNAs, and are capable of generating proteins when isolated from the cell body (for review see [1]). Nevertheless, little is known about the mechanisms that are used to bring specificity to the axon’s protein synthesis apparatus.

It is appealing to hypothesize that axons maintain multiple levels of translational regulation to temporally match the synthesis of new proteins to the physiological needs of this subcellular domain. RNA profiles of axons and dendrites have shown that an increasingly complex fraction of the neuron’s transcriptome can localize into these processes [2,3]. The 3'UTRs of mRNAs have most often been linked to subcellular localizing activity, including localization into axons [4]. Localization of the mRNAs is driven by RNA binding proteins that recognize cis-elements in the mRNAs [4]. For some localizing mRNAs, the same RNA binding protein that is needed for the mRNA’s transport to subcellular sites regulates translation. For example, ZBP1 inhibits translation of its cargo mRNAs and this translational repression is released upon phosphorylation of ZBP1 [5]. Some guidance cues have been shown to trigger phosphorylation of ZBP1 in axons, presumably allowing stimulus-dependent translation of the ZBP1 mRNA cargo [6]. For other axonal mRNAs, the mechanisms for translational silencing are unknown, and there are now several examples of localized mRNAs that are stored until needed. Importin β1, RanBP1, and vimentin mRNAs are stored in axons until needed, allowing stimulus-dependent translation of the ZBP1 mRNA cargo [7–9]. Though the molecular mechanisms are still unclear, these observations indicate that mRNA transport and local translational activation of the mRNA are not always mechanistically linked.

Initiation of translation is the rate-limiting step of protein synthesis in eukaryotes. Initiating translation requires a set of specialized proteins known as initiation factors (eIFs) that recruit the 40S ribosome subunit to the m7GpppN structure or m7GTP residue (or ‘cap’) located at the immediate 5'-end of most eukaryotic mRNAs [10]. The cap-dependent initiation complex then scans the 5'-untranslated regions (UTR) until finding an AUG initiation codon in the appropriate context to start protein synthesis [11]. In contrast to the general cap-dependent mechanism of protein synthesis, some viral RNAs have been shown to initiate translation through an alternate mechanism driven by internal ribosome entry sites (IRES) [12,13]. These cis-acting...
5' UTR elements form secondary and tertiary RNA structures that can recruit the translational machinery to an internal position in the mRNA. This allows the ribosome to bypass stable RNA structures in the 5' UTRs and internally initiate translation of the mRNA [14]. This has also been shown to occur for some cellular mRNAs, and cap-independent protein synthesis can provide a selective advantage for generating new proteins under conditions when traditional cap-dependent translation is compromised [15]. Cap-independent mRNA translation has been demonstrated in dendrites [16,17], but the possibility that cellular mRNAs transported into axons from the cell body can be translated through internal initiation of translation has not been tested.

We previously showed that calreticulin mRNA contains two 3' UTR cis-elements that confer subcellular localization; a proximal RNA element for stimulus-dependent transport requiring activation of e-Jun N-terminal kinase (JNK) pathways and a second more distal element for constitutive transport into axons [18]. The 5' UTR of calreticulin confers translational regulation in response to lysophosphatic acid (LPA) through phosphorylation of eIF2α [19]. Thus, the axonal transport and localized translation of calreticulin mRNA can be through distinct mechanisms. Here we show that, despite this activation of axonal calreticulin mRNA translation upon inhibition of eIF2α, calreticulin’s 5' UTR has no IRES activity in sensory neurons, However, axonal processes are capable of cap-independent translational regulation since both viral RNA and grp78/BiP mRNA 5' UTRs function as IRESs when placed between cistrons of axonally targeted bicistronic reporter mRNAs.

## Results

We previously showed that the mRNA encoding the ER chaperone protein calreticulin localizes to the axons of DRG and cortical neurons through cis-elements in its 5' UTR [18]; Synthesis of calreticulin protein is increased by release of ER Ca$^{2+}$ stores in isolated sensory axons [20]. This translational control of axonal calreticulin mRNA is conveyed by its 5' UTR [19]. The 5' UTR of rat calreticulin shows high primary sequence identity between vertebrates, but it is short (63 nucleotides) and has a single AUG initiation codon. Despite this relatively short length, this 5' UTR has no IRES activity in sensory neurons. However, axonal processes are capable of cap-independent translational regulation since both viral RNA and grp78/BiP mRNA 5' UTRs function as IRESs when placed between cistrons of axonally targeted bicistronic reporter mRNAs.

IRES-mediated translation depends on availability of IRES trans-acting factors (ITAFs) that modulate IRES activity [22,25]. Consequently, activity of individual IRES elements can vary considerably from one cell type to another [24,25]. In a recent study, we saw differential translational regulation of calreticulin mRNA in axons compared to neuronal cell bodies [19]. Thus, we asked if the 5' UTR of calreticulin might have IRES activity in axons. For this, we generated a bicistronic vector with the diffusion-limited reporters mCherrymyr and eGFPMyr to visualize sites of protein synthesis in neuronal processes (Fig. 1A) and the 5' UTR of calreticulin mRNA for axonal targeting [18]. Similar to the HEK cells, the 5' UTR of calreticulin showed no evidence of IRES activity when placed between cistrons of the axonally targeted bicistronic mCherrymyr5' Cal-eGFPMyr3' Cal mRNA. Although mCherry signals were clearly visible in the axons and cell body of the DRG neurons, neither the axons nor cell body showed any GFP fluorescence (Fig. 2A). FRAP analyses showed significant recovery of the axonal mCherry fluorescence after photobleaching that was prevented by pretreatment with translational inhibitors (Fig. 3; Videos S1, S2). Thus, the mCherrymyr5' Cal-eGFPMyr3' Cal construct generates an mRNA that can be used to locally synthesize mCherrymyr through cap-dependent translation but does not support cap-independent translation of the downstream eGFPMyr cistron.

Although IRES-dependent translation has been documented in dendrites [17], axonal processes have never been tested for this ability. To address the question of IRES-dependent translation in axons, we initially asked if the well-characterized EMCV IRES could support internal translational initiation in axons. For this, we substituted the EMCV IRES sequence for the calreticulin 5' UTR in pmCherrymyr5' Cal-eGFPMyr3' Cal to generate a bicistronic construct encoding mCherrymyrEMCV-eGFPMyr3' Cal mRNA. In contrast to pmCherrymyr5' Cal-eGFPMyr3' Cal transfections, eGFP fluorescence was clearly visible in the cell bodies and axons of DRG neurons expressing mCherrymyrEMCV-eGFPMyr3' Cal mRNA (Fig. 2B). FRAP analyses of distal axons showed that the axonal mCherry and eGFP fluorescence recovered from photobleaching (Fig. 4A; Video S3). Moreover, recovery of the mCherry and eGFP fluorescence was attenuated by anisomycin and cycloheximide (Fig. 4B, Video S4). Since these agents block distinct steps of translational elongation [26,27], these data emphasize that the recovery of eGFP fluorescence in the axons is undoubtedly through internally initiated translation of the axonal mCherrymyrEMCV-eGFPMyr3' Cal mRNA. Thus, it appears that sensory axons tested here do have the ability to internally initiate translation through the EMCV IRES element.

Since the DRG neurons showed capacity for internal initiation of a viral RNA IRES element but not of the calreticulin’s mRNA’s 5' UTR, we asked if other cellular mRNAs might show IRES activity in the axonal compartment. grp78/BiP was the first cellular mRNA demonstrated to have cap-independent translation [28]. Similar to calreticulin mRNA, grp78/BiP mRNA encodes an ER chaperone protein that is locally translated in sensory axons [29,30]. Thus, we generated an axonally targeting bicistronic construct with the 5' UTR of rat grp78/BiP in the intercistronic region of mCherrymyr and eGFPMyr (mCherrymyr5' BiP-eGFPMyr3' Cal) to test for IRES activity in the DRG axons. Robust mCherry and eGFP signals were seen in the cell body and axons of mCherrymyr5' BiP-eGFPMyr3' Cal expressing neurons (Fig. 2C). Both mCherry and eGFP fluorescence in the distal axons of mCherrymyr5' BiP-eGFPMyr3' Cal transfected neurons recovered from photobleaching over a time course paralleling what we have previously demonstrated for axonally generated reporter proteins (Fig. 5; Video S5) [9,18]. This recovery of axonal reporter
fluorescence after photobleaching was also blocked by anisomycin and cycloheximide (Fig. 5B; Video S6). Thus, the axonal compartment of the adult rodent sensory neurons used here has the capacity to internally initiate translation through BiP IRES element but not through calreticulin 5'UTR. We recently showed that the 5'UTR of calreticulin mRNA confers local translational activation by lysophosphatidic acid (LPA) [19]. However, the mCherry-myr5'Cal-GFPmyr3'Cal expressing neurons did not show any detected GFP fluorescence, neither in axons or cell body, after 2 hours LPA treatment (Fig. S1).

Discussion

Localized synthesis of neuronal proteins contributes to axonal responses to guidance cues and injury [31]. Several lines of evidence suggest that the axonal translational machinery can match the translation of different mRNAs to the stimulus provided. For example, positive and negative guidance cues have been linked to translation of different axonal mRNAs [32–34]. Additionally, nerve injury triggers translation of axonal mRNAs whose encoded proteins are needed to stimulate transcriptional responses in the neuronal cell body [7–9,35]. Recent work has shown that phosphorylation of the RNA binding protein ZBP1 regulates the translation of specific axonal mRNAs [6,36]. However, only a few axonal mRNAs are known targets of ZBP1 and there are likely to be many axonal mRNAs that are not regulated by ZBP1. Consistent with this, we recently showed that calreticulin mRNA studied here is not a target for ZBP1 binding in axons [36]. With hundreds of other mRNAs localizing into axons, it is likely that axons have multiple mechanisms at their disposal for temporally regulating translation of different axonal mRNA cohorts.

The data shown here indicate that at least some axonal mRNAs can be translated using internal initiation elements. It is likely that other polarized cells that make use of localized protein synthesis will utilize cap-independent translation to locally regulate synthesis of proteins.

Translation through IRES elements was initially recognized in viral RNAs, where viral proteins inactivate cap-dependent translation favoring translation of viral RNAs without the need for the eIF4 cap-binding complex [12,13]. Cap-dependent translation can be down regulated during development, stress, and disease states such that internal initiation is needed for continued protein expression [37,38]. This brings a level of specificity to the translational apparatus to preferentially generate proteins needed under unique physiological states. Using FRAP to detect axonally translated reporter proteins, our studies show that both viral and cellular RNA elements previously shown to have IRES activity in other cellular systems provide internal initiation of translation in sensory axons. In previous FRAP studies focusing on cis-elements for axonal RNA transport, both isolated axons and repetitive photobleaching of proximal axonal segments was used to rule out the possibility that cell body-derived reporter protein accounted for the rapid recovery of fluorescence [9]. In our hands, this recovery from photobleaching in distal axons has consistently correlated with axonal localization of the reporter mRNAs [18,35]. In contrast, we are able to fully differentiate RNA transport and translational control with the bicistronic reporters used here, since the neurons expressing axonally targeted

Figure 1. 5'UTR of calreticulin mRNA does not show IRES activity in HEK-293 cells. A. Schematic of monocistronic and bicistronic constructs used for mRNA expression in HEK cells and DRG neurons is shown. 5' CAL and 3' CAL correspond to 5' and 3' UTRs of rat calreticulin mRNA. 5'BIP corresponds to the 5'UTR of grp78/Bip mRNA. EMCV corresponds to the 5' leader sequence of the encephalomyocarditis virus RNA. The constructs used in HEK cells contained standard mCherry and eGFP, while those for neuronal transfections contained diffusion limited mCherrymyr and eGFPmyr reporters. The neuronal constructs also included an axonal targeting 3'UTR (3'Cal). B-D, Representative exposure matched fluorescent images are shown for HEK-293 cells, 48 h post-transfection, for bicistronic pmCherry-5'Cal-eGFP (B), monocistronic ps'Cal-eGFP (C) and bicistronic pmCherry-EMCV-eGFP (D) [scale bars = 200 μm].
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Figure 2. Bicistronic reporters support IRES-dependent translation in sensory neurons. Representative static images of DRG neurons transfected with bicistronic pmCherry<sup>myr5′</sup>Cal-eGFP<sup>myr3′</sup>Cal (A), pmCherry<sup>myr</sup>EMCV-eGFP<sup>myr3′</sup>Cal (B), and pmCherry<sup>myr5′</sup>BiP-eGFP<sup>myr3′</sup>Cal (C) reporters are shown at 48 h post-transfection. Both mCherry (red, left panel) and eGFP (green, right panel) fluorescence is seen in the cell bodies and
mCherry<sup>5′</sup>-Cal-eGFP<sup>3′</sup> mRNA showed recovery of mCherry.

The EMCV IRES used here is a highly structured element present in the 5′UTR region of its positive single strand RNA [12]. This RNA structure is commonly used in commercial bicistronic vectors [39]. Activity of the EMCV IRES in the sensory axons shown here may have important implications for effects of viral infection on mature neurons. For example, IRES of other picornaviruses such as poliovirus can contribute to the neurovirulence of different viruses [40]. Recently, the Jaffrey group showed evidence for IRES-driven translation through EMCV IRES with static imaging approaches applied to axons transduced with a modified Sindbis RNA virus [41]. Our study advances their observations by showing that a bicistronic mRNA can be transported into axons from the cell body and locally translated through cap-independent mechanisms. Moreover, we show that an axonally transported cellular mRNA, grp78/BiP, has localized IRES activity in axons. The 5′UTR of grp78/BiP has similarly been shown to have IRES activity in other cellular systems [28,42]. Heat shock was shown to drive translation through the 5′UTR of this Ca<sup>2+</sup>-binding ER chaperone protein [21]. Both transcription and translation of grp78/BiP and other chaperone proteins has been shown to be increased with other forms of cellular stress that lead to the unfolded protein response [43]. We have previously shown that the 3′UTR of grp78/BiP mRNA is sufficient for its transport into axons [18]. Although it is not clear what cellular mechanisms trigger cap-independent translation of this mRNA, our data suggest that grp78/BiP mRNA translation in axons can be regulated through RNA element(s) distinct from those used for targeting the mRNA for transport into axons. Local siRNA-mediated depletion of mRNAs from axons of cultured neurons and in peripheral nerve in vivo shows that axons have RNA interference machinery [44,45]. Moreover, microRNAs were detected in sympathetic axons in culture and shown to block translation of axonal CoxIV mRNA [46,47]. Thus, non-coding mRNAs may offer a means for translational specificity beyond the choice between cap-dependent and IRES-mediated translation that we have tested here.

In summary, we show that a bicistronic mRNA can be transported into the axonal compartment and locally translated through cap-dependent and cap-independent mechanisms. This provides evidence for multiple mechanisms of translational control for axonal mRNAs. Metabolic labeling of axons indicated that only 5–10% of total cellular protein synthesis occurs in the axons [48,49]. For the cap-independent translation seen here, both viral and cellular RNAs are functional IRES elements in axons similar to what has been documented for studies in whole cells. Efficiency of different IRES elements has been reported to be cell type specific possibly due to the availability of cellular factors required for a particular IRES [22,50]. Further studies will be needed to understand how IRES-dependent translation is mediated in axons. Nonetheless, our studies indicate that distinct mechanisms have evolved to modulate translation of axonal localized mRNAs.

**Materials and Methods**

**Cell Culture and Transfections**

HEK-293 cells were maintained in 10% fetal bovine serum supplemented DMEM at 37°C and 5% CO<sub>2</sub> on plastic dishes. HEK-293 cells were transfected with 2 μg plasmid using Lipofectamine 2000 Reagent per manufacturer’s instructions (Invitrogen). Transfected cells were cultured for 48 h on glass coverslips.

Primary cultures of L4–5 dorsal root ganglion (DRG) were prepared from adult Sprague Dawley rats (175 g). These were dissociated using 36 mg/ml collagenase type XI (Sigma) for

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**Figure 3. 5′ UTR of calreticulin does not support cap-independent translation in sensory neurons.**

A, Representative time-lapse sequences from FRAP analyses of DRG neurons transfected with pmCherry<sup>5′</sup>-Cal-eGFP<sup>3′</sup> are shown. mCherry fluorescence is displayed as a spectrum with white being the brightest signal as indicated. The white boxed regions represent the regions subjected to photobleaching and the arrows indicate the regions of the terminal axon where recovery was quantified. The upper row shows cultures standard medium and lower row shows cultures pretreated with 150 μM anisomycin [scale bar = 50 μm]. B, Quantification of normalized mCherry fluorescence intensity in ROI as indicated from multiple FRAP sequences are shown. Signals are shown as the average percent of pre-bleach levels ± SEM (n ≥ 12 over at least 4 independent transfections; *** = p<0.001 by two-way ANOVA comparing conditions to t = 0 min for indicated time points).

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30 min. The dissociated DRGs were transfected using AMAX1 Nucleofector apparatus with the SCV Nucleofector kit (program G-8; Lonza, Inc.). Cells were then resuspended in DMEM/F12 (Mediatech) with 10% horse serum (Hyclone) and cultured for 48–72 h. For DRGs, medium was replaced at 20 h post-transfection and 10 μM arabinofuranosyl cytidine (Sigma) was included to decrease proliferation of non-neuronal cells.

DNA Expression Constructs

All constructs used for expression in HEK cells were generated from the pBMN vector backbone (provided by Dr. Luis Sigal). p5’-Cal-mCherrymyr3’-Cal was used as a template for PCR to generate 5’UTR rat calreticulin (GenBank Accession, NM_022399) with NotI and NcoI restriction sites for subcloning upstream of the eGFP coding sequence in pBMN. This generated the monocistronic construct 5’-Cal-eGFP, mCherry was then amplified from p5’-Cal-mCherrymyr3’-Cal incorporating BamHI and NcoI restriction sites for subcloning upstream of the above 5’UTR calreticulin to generate the bicistronic construct pmCherry-5’-Cal-eGFP. The same mCherry PCR product was cloned upstream of the EMCV IRES sequence (NC_001479; IRESite Id: 140) in the pBMN vector to generate the bicistronic construct pmCherry-EMCV-eGFP (see Fig. 1A). In each case where PCR products were used for this and subsequent vector production, inserts were fully verified by sequencing.

All the bicistronic constructs for testing axonal translation in DRG neurons were generated in the pcDNA3.1 backbone (Invitrogen). For this, we used eGFP and mCherry with an N-terminal myristylation signals (eGFPmyr and mCherrymyr, respectively). The original eGFPmyr vector with 5’ and 3’ UTRs of Cal-myrrc was obtained from Dr. Erin Schuman [51]; the mCherrymyr was generated by PCR [19]. The diffusion-limited bicistronic vectors were prepared in two consecutive steps. First, a monocistronic construct pcDNA3.1-mCherrymyr was generated by cloning BamHI/NotI fragment of mCherrymyr from p5’-Cal-mCherrymyr3’-Cal construct [19] into pcDNA3.1. 5’Cal-eGFP-myrr3’-Cal insert was then isolated by EcoRV/Xhol digest of p5’-Cal-eGFPmyr3’-Cal and subcloned downstream of mCherrymyr in pcDNA3.1-mCherrymyr to yield the bicistronic construct pmCherrymyr3’-Cal-eGFPmyr3’-Cal (Fig. 1A).

For the EMCV IRES and grp78/BiP 5’UTR bicistronic constructs, an intermediate pmCherrymyr-eGFPmyr3’-Cal was generated such that EcoR1 and Xhol restriction sites were inserted for cloning purposes between the mCherrymyr and eGFPmyr open reading frames. cDNA for the EMCV IRES (NC_001479; IRESite Id: 140) was generated by PCR from pBMN backbone. EcoR1/Xhol digested EMCV IRES PCR product was subcloned to generate pmCherrymyr-EMCV-GFPmyr3’-Cal. Then grp78/BiP IRES insert (GenBank Accession X87949.1; IRESite Id: 570) was isolated from an EcoRV/Xhol digest of pUC57-BiP (GenScript) and subcloned between the mCherrymyr and eGFPmyr cistrons to generate pmCherrymyr3’-BiP-eGFPmyr3’-Cal (Fig. 1A).

Statistical Analyses

Data were analyzed using GraphPad Prism 5 software package. Two-way ANOVA followed by Bonferroni post-hoc multiple comparisons were used to compare the time for the recovery between treatments at each time point. All values were expressed as mean ± standard error of the mean (SEM). P values of ≤0.05 were considered as significant.
Figure 5. 5' UTR of grp78/BiP mRNA can function as an IRES in axons. A-B, Representative time-lapse sequences from FRAP analyses of DRG neurons transfected with pmCherry^{myr5} BiP-eGFP^{myr3} Cal as in Fig. 4 are shown. Sequences for cap-dependent translation of mCherry are shown in A and for IRES-dependent translation of eGFP are shown in B. The upper rows for A and B show cultures standard medium and lower rows show IRES Translation in Axons
Supporting Information

Figure S1  LPA effect in 5′UTR cap-independent translation. Representative images of DRG neurons expressing indicated bicistronic mRNAs are shown after 2 h exposure to 30 μM LPA. Only the mCherry signal is seen for the mCherrymyr5-Cal-eGFPmyr-3‘Cal expressing neurons, both in cell body (arrowhead) and axons (arrow). These data indicate LPA does not trigger cap-independent translation through calreticulin’s 5′UTR in sensory neurons [scale bars = 50 μm].

Video S1  Recovery of axonal mCherry fluorescence in mCherrymyr5-Cal-eGFPmyr mRNA expressing DRG neurons. Representative FRAP sequence of a neuron transfected with pmCherrymyr5-Cal-eGFPmyr-3‘Cal is shown with fluorescent intensity shown as a spectrum as outlined in Figure 3 (original time lapse = 30 min, with 2 min pre-bleach and 28 min post-bleach at 1 frame/min) [scale bar = 50 μm].

Video S2  Protein synthesis inhibition attenuates axonal recovery of cap-dependent translation in mCherrymyr5-Cal-eGFPmyr mRNA expressing DRG neurons. Representative FRAP sequence as in Video S1 except cultures were pretreated with 150 μM anisomycin prior to photobleaching (original time lapse = 30 min, with 2 min pre-bleach and 28 min post-bleach at 1 frame/min) [scale bar = 50 μm].

Video S3  Recovery of axonal eGFP fluorescence in mCherrymyrEMCV-eGFPmyr mRNA expressing DRG neurons. Representative FRAP sequence of a neuron transfected with pmCherrymyrEMCV-eGFPmyr-3‘Cal is shown as outlined in Figure 4 with fluorescent intensity shown as a spectrum as outlined (original time lapse = 30 min, with 2 min pre-bleach and 28 min post-bleach at 1 frame/min) [scale bar = 50 μm].

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Author Contributions

Performed the experiments: AP. Analyzed the data: AP JLT. Wrote the paper: AP JLT.
16. Dobson T, Minic A, Nielsen K, Amiot E, Krushel I (2005) Internal initiation of translation of the Trkβ mRNA is mediated by multiple regions within the 5′ leader. Nucleic Acids Res 33: 2929–2941.

17. Pinkstaff JK, Chappell SA, Mauro VP, Edelman GM, Krushel LA (2001) Internal initiation of translation of five dendritically localized neuronal mRNAs. Proc Natl Acad Sci U S A 98: 2770–2775.

18. Vuppalaunchi D, Coleman J, Yoo M, Merianda TT, Yadhati AG, et al. (2010) Conserved 3′-untranslated region sequences direct subcellular localization of chaperone protein mRNAs in neurons. J Biol Chem 285: 18025–18036.

19. Vuppalaunchi D, Merianda TT, Donnelly C, Williams G, Yoo S, et al. (2012) Lyso phosphatidic acid differentially regulates axonal mRNA translation through 5′ UTR elements. Mol Cell Neurosci. in press.

20. Wang W, van Nierkerk E, Willis DE, Twiss JL (2007) RNA transport and localized protein synthesis in neurological disorders and neural repair. Dev Neurobiol 67: 1166–1182.

21. Kim YK, Jang SK (2002) Continuous heat shock enhances translational initiation directed by internal ribosomal entry site. Biochem Biophys Res Commun 297: 224–231.

22. Komar AA, Hatzoglou M (2011) Cellular IRES-mediated translation: the war of ITAFs in pathophysiological states. Cell Cycle 10: 229–240.

23. Pacheco D, Martinez-Salas E (2010) Insights into the biology of IRES elements through riboproteomic approaches. J Biomed Biotechnol 2010: 458927.

24. Borman AM, Le Mercier P, Girard M, Kean KM (1997) Comparison of picomaviral IRES-driven internal initiation of translation in cultured cells of different origins. Nucleic Acids Res 25: 925–932.

25. Licurci M, Christian SL, Pongpootpant T, Hirasawa K (2011) In vitro and in vivo comparison of viral and cellular internal ribosome entry sites for bicistronic vector expression. Gene Ther 18: 631–636.

26. Gale EF (1981) The Molecular basis of antibiotic action. London: Wiley.

27. Grollman AP (1967) Inhibitors of protein biosynthesis. II. Mode of action of anisomycin. J Biol Chem 242: 3226–3233.

28. Macejak DG, Sarnow P (1991) Internal initiation of translation mediated by the leader of a cellular mRNA. Nature 353: 90–94.

29. Willis D, Li KW, Zheng JQ, Chang JH, Smit A, et al. (2005) Differential transport and local translation of cytoskeletal, injury-response, and neurodegeneration protein mRNAs in axons. J Biol Chem 280: 770–791.

30. Willis DE, van Nierkerk EA, Sasaki Y, Mesngon M, Merianda TT, et al. (2007) Extracellular stimuli specifically regulate localized levels of individual neuronal mRNAs. J Cell Biol 170: 965–980.

31. Donnelly CJ, Fainzilber M, Twiss JL (2010) Subcellular communication through RNA transport and localized protein synthesis. Traffic 11: 1498–1505.

32. Leung KM, van Hoek FP, Lin AC, Allison R, Standart N, et al. (2006) Asymmetrical beta-actin mRNA translation in growth cones mediates attractive turning to netrin-1. Nat Neurosci 9: 1247–1256.

33. Wu KY, Hengst U, Cox LJ, Makoosco EZ, Jeromein A, et al. (2005) Local translation of RhoA regulates growth cone collapse. Nature 436: 1020–1024.

34. Yao J, Sasaki Y, Wen Z, Bassell GJ, Zheng JQ (2006) An essential role for beta-actin mRNA localization and translation in Ca2+-dependent growth cone guidance. Nat Neurosci 9: 1265–1273.

35. Ben-Yakov K, Dagan SY, Segal-Ruder Y, Shalem O, Vuppalaunchi D, et al. (2012) Axonal transcription factors signal retrogradely in lesioned peripheral nerve. EMBO J.

36. Donnelly CJ, Willis DE, Xu M, Top C, Jiang C, et al. (2011) Limited availability of ZBP1 restricts axonal mRNA localization and nerve regeneration capacity. EMBO J 30: 4665–4677.

37. Le Quesne JP, Spriggs KA, Bushell M, Willis AE (2010) Dysregulation of protein synthesis and disease. J Pathol 220: 140–151.

38. Spriggs KA, Stoneley M, Bushell M, Willis AE (2008) Re-programming of translation following cell stress allows IRES-mediated translation to predominate. Biol Cell 100: 27–38.

39. Bochko YA, Pahneberg AC (2006) Translational efficiency of EMCV IRES in bicistronic vectors is dependent upon IRES sequence and gene location. Biotechniques 41: 283–294, 286, 288 passim.

40. Groemer M, Bossert B, Arita M, Nomoto A, Wimmer E (1999) Dual stem loops within the poliovirus internal ribosomal entry site control neurovirulence. J Virol 73: 958–964.

41. Walker BA, Hengst U, Him JH, Jean NL, Schmidt EF, et al. (2012) Reprogramming axonal behavior by axon-specific viral transduction. Gene Ther.

42. Sarnow P (1989) Translation of glucose-regulated protein 78/immunoglobulin heavy-chain binding protein mRNA is increased in poliovirus-infected cells at a time when cap-dependent translation of cellular mRNAs is inhibited. Proc Natl Acad Sci U S A 86: 5795–5799.

43. Brostrom MA, Brostrom CO (2005) Calcium dynamics and endoplasmic reticular function in the regulation of protein synthesis: implications for cell growth and adaptability. Cell Calcium 34: 345–363.

44. Hengst U, Cox LJ, Makoosco EZ, Jaffrey SR (2006) Functional and selective RNA interference in developing axons and growth cones. J Neurosci 26: 5727–5732.

45. Murashov AK, Chintalagurtu V, Islamov RR, Lever TE, Pak ES, et al. (2007) Poliovirus RNAi pathway is functional in peripheral nerve axons. FASEB J 21: 636–670.

46. Aschrafi A, Schwecheter AD, Maneza MG, Natera-Naranjo O, Gisio AE, et al. (2008) MicroRNA-338 regulates local cytochrome c oxidase IV mRNA levels and oxidative phosphorylation in the axons of sympathetic neurons. J Neurosci 28: 12581–12590.

47. Natera-Naranjo O, Aschrafi A, Gisio AE, Kaplan BB (2010) Identification and quantitative analysis of microRNAs located in the distal axons of sympathetic neurons. RNA 16: 1516–1529.

48. Hengst U, Cox LJ, Makoosco EZ, Jaffrey SR (2006) Functional and selective RNA interference in developing axons and growth cones. J Neurosci 26: 5727–5732.

49. Lee SK, Hollenbeck PJ (2003) Organization and translation of mRNA in neuronal axons. J Neurosci 19: 1–9.

50. Eng H, Lund K, Campenot RB (1999) Synthesis of beta-tubulin, actin, and other proteins in axons of sympathetic neurons in compartmented cultures. J Neurosci 19: 1–9.

51. Aakalu G, Smith WB, Nguyen N, Jiang C, Schuman EM (2001) Dynamic visualization of local protein synthesis in hippocampal neurons. Neuron 30: 489–502.