Internal initiation of translation of five dendritically localized neuronal mRNAs

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In neurons, translation of dendritically localized mRNAs is thought to play a role in affecting synaptic efficacy. Inasmuch as components of the translation machinery may be limiting in dendrites, we investigated the mechanisms by which translation of five dendritically localized mRNAs is initiated. The 5′ leader sequences of mRNAs encoding the activity-regulated cytoskeletal protein, the α subunit of calcium–calmodulin-dependent kinase II, dendrin, the microtubule-associated protein 2, and neurogranin (RC3) were evaluated for their ability to affect translation in the 5′ untranslated region of a monocistronic reporter mRNA. In both neural and nonneural cell lines, the activity-regulated cytoskeletal protein, microtubule-associated protein 2, and α-CaM Kinase II leader sequences enhanced translation, whereas the dendrin and RC3 5′ untranslated regions slightly inhibited translation as compared with controls. When cap-dependent translation of these constructs was suppressed by overexpression of a protein that binds the cap-binding protein eIF4E, it was revealed that translation of these mRNAs had both cap-dependent and cap-independent components. The cap-independent component was further analyzed by inserting the 5′ leader sequences into the intercistronic region of dicistronic mRNAs. All five leader sequences mediated internal initiation via internal ribosome entry sites (IRESes). The RC3 IRES was most active and was further characterized after transfection in primary neurons. Although translation mediated by this IRES occurred throughout the cell, it was relatively more efficient in dendrites. These data suggest that IRESes may increase translation efficiency at postsynaptic sites after synaptic activation.

In neurons, a number of mRNAs are transported into dendrites (1) and translated locally (2). Ribosomes and other components of the translation machinery are found within dendritic processes (3, 4), although at levels that are low compared with the cell body (5), and recent studies have shown that *de novo* protein synthesis within dendrites is required for lasting changes in synaptic efficacy in response to neural activity or particular neurotrophins (6, 7). Moreover, many dendritically localized mRNAs encode proteins that are critical for certain forms of synaptic plasticity (8). These findings suggest that translation within dendrites may provide a mechanism for the rapid local synthesis of proteins in response to certain synaptic stimuli (9–11).

In eukaryotes, the translation of most mRNAs is thought to occur after the recruitment of the translation machinery at the m7GpppN cap structure found at the 5′ end of the mRNA, a process that requires the cap-binding protein eukaryotic initiation factor 4E (eIF4E). eIF4E is rate limiting in the perikarya for cap-dependent translation (12) and is likely to be present at a relatively low concentration in dendrites. It is conceivable that cap-dependent mechanisms may not be sufficient to ensure adequate translation initiation of mRNAs that are localized in dendrites. We therefore investigated the possibility that the translation of dendritically localized mRNAs might also involve cap-independent internal initiation.

In the present study, we identify internal ribosome entry sites (IRESes) within the 5′ leader sequences of five dendritically localized mRNAs: those for the activity regulated cytoskeletal protein (ARC), the α subunit of calcium–calmodulin-dependent kinase II (αCaM Kinase II), dendrin, the microtubule-associated protein 2 (MAP2), and neurogranin (RC3). For RC3, we demonstrate that internal initiation occurs to a relatively greater extent in dendrites than in the cell body and that both cap-dependent and cap-independent mechanisms contribute to the translation of these mRNAs in neurons. These five IRESes may enhance translation in dendrites by increasing the efficiency with which they recruit translation machinery. Such IRESes may be involved in the local regulation of activity-dependent protein synthesis by enabling efficient translation under conditions that inhibit cap-dependent translation, for example, conditions following synaptic input that may lead to a decrease in cap-dependent translation (13–15).

Materials and Methods

Constructs Used to Evaluate IRES Activity. Full-length 5′ leader sequences from the transcription start site, up to but not including the AUG initiation codon, were obtained from the αCaM Kinase II, dendrin, ARC, MAP2, and RC3 mRNAs by reverse transcription–PCR by using adult rat hippocampal mRNA. These sequences were cloned into the *Eco*RI and *Nco*I restriction sites contained within the intercistronic region of the *RP* (*Renilla, Photinus* or *RP*h (*Renilla, Photinus*, hairpin) dicistronic vectors (16, 17) that encode *Renilla* and *Photinus* luciferase proteins as the first and second cistrons, respectively.

Monocistronic luciferase constructs were generated from the EYFP-N1 (CLONTECH) vector by replacing the gene-encoding enhanced yellow fluorescent protein (EYFP) with the *Photinus* luciferase gene from the *RP* constructs by using the *Eco*RI and *Xba*I restriction sites. The chloramphenicol acetyl transferase (CAT) gene was used as a control for transfection efficiency. A blunt-ended *Bam*HI/*Bgl*II fragment containing the simian virus 40 promoter, the CAT reporter gene, and polyadenylation sequences from pCAT3 (Promega) was cloned into the *Ase*I restriction site of these constructs after fill in of 3′ recessed ends by treatment with the Klenow fragment of DNA polymerase. An EYFP construct, designated EYFP/NLS, was used to test the ability of the RC3 3′ untranslated region (UTR) to target mRNA to dendrites. This construct contains a nuclear localization signal (NLS) and a nuclear matrix-binding site attached to the EYFP coding sequence to limit diffusion of EYFP from the cell body to the dendrites. For *in situ* analysis of IRES activity, a dicistronic

Abbreviations: eIF4E, eukaryotic initiation factor 4E; IRES, internal ribosomal entry site; ARC, activity-regulated cytoskeletal protein; αCaM kinase II, α subunit of calcium–calmodulin-dependent kinase II; MAP2, microtubule-associated protein 2; RC3, neurogranin; EYFP, enhanced yellow fluorescent protein; CAT, chloramphenicol acetyltransferase; UTR, untranslated region; NLS, nuclear localization signal; ECFP, enhanced cyan fluorescent protein; *P:R*, ratio of *Photinus* to *Renilla* luciferase activities; *P*M, Photinus Monocistronic; EMCV, encephalomyocarditis virus.

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fluorophore vector was constructed that encodes enhanced cyan fluorescent protein (ECFP) as the first cistron and EYFP as the second cistron. Both fluorescent cistrons were modified to contain the mouse ornithine decarboxylase destabilizing motif, which shortened the half-lives of these proteins to approximately 4 h. This motif was obtained from the pd4 EGFP-N1 vector (CLONTECH) as a *Bsr*GI/*Not*I fragment. mRNA expressed from this construct was targeted to dendrites by using the 3′ UTR from the RC3 gene.

Cell Culture. The tissue culture cell lines used in this study were rat neural tumor B104, mouse neuroblastoma N2a, rat glial tumor line C6, mouse fibroblast NIH 3T3, and mouse fibroblast LMTK$^-$. Cells were prepared and transiently transfected as described (17). Primary cultures of dissociated hippocampal neurons were cultured on poly-L-lysine-coated chamber slides, as described (18). Culture media were exchanged twice weekly until neurons had reached maturation (21–25 days *in vitro*). Hippocampal neurons were transfected by using calcium phosphate (Promega).

Reporter Gene Assays. Transiently transfected cells were assayed for both *Renilla* and *Photinus* luciferase activities (17). Luciferase activities generated by these mRNAs were expressed as the ratio of *Photinus* to *Renilla* luciferase activities (*P*:*R*) and were normalized to the activity of the parent vector. All experiments were performed at least three times, and individual transfections were performed in triplicate. CAT enzyme activity was measured by liquid-scintillation counting according to the manufacturer's protocol (Promega). In experiments using a hypophosphorylated form of 4E-BP1 (containing Thr-37-Ala/Thr-46-Ala mutations), plasmids expressing this protein or the parent vector (both based on pACTAG-2) were cotransfected with the monocistronic constructs described above, using a 6-fold molar excess of the 4EBP1 or control expression constructs (19). 4E-BP1 double mutant and control expression plasmids were kindly provided by Nahum Sonnenberg (McGill University, Montreal).

RNA and *in Situ* Hybridization Analysis. The coding regions of the *Photinus* luciferase and EYFP genes were subcloned into the PBS SK II (+) vector (Stratagene). Linearized plasmids were used as templates for *in vitro* transcription to generate both ^{32}P-radiolabeled and digoxygenin-labeled cRNA probes for Northern blots and *in situ* hybridization, which were performed as previously described (20, 21).

Fluorescent Microscopic Analysis. Transfected neurons were fixed by using 4% paraformaldehyde in phosphate-buffered solution, placed under coverslips with Slow-Fade reagent (Molecular Probes), and visualized and quantified as described previously (22). Images were captured from each fluorescent filter channel for equal time periods and optimized so that the emission from both fluorophores was within a linear range. At least 20 images were quantified for each construct.

Statistical Analyses. Experimental data were analyzed for statistical significance by using a one-way ANOVA followed by an individual analysis of experimental vs. control data points by using a two-tailed Student's *t* test. Results were considered significant at $P < 0.05$. Bar graphs represent the average of data points with error bars representing SEM.

Results

Leader Sequences from Dendritically Localized mRNAs Use both Cap-Dependent and Cap-Independent Mechanisms to Initiate Translation. To determine the effect on translation of the full-length leader sequences of RC3, ARC, dendrin, αCaM kinase II (CamK IIa), and MAP2 mRNAs, they were inserted into the 5′ UTR of a monocistronic reporter mRNA [Photinus Monocistronic (*P*M)] encoding the *Photinus* luciferase protein and examined for luciferase activity (Fig. 1*A*). The translation of β-globin mRNA has been shown to be cap-dependent (23), and the 5′ UTR of this mRNA was included in these studies as a control. Monocistronic constructs were transfected into B104 cells. RNA blot analyses of total RNA from transfected B104 cells showed a similar

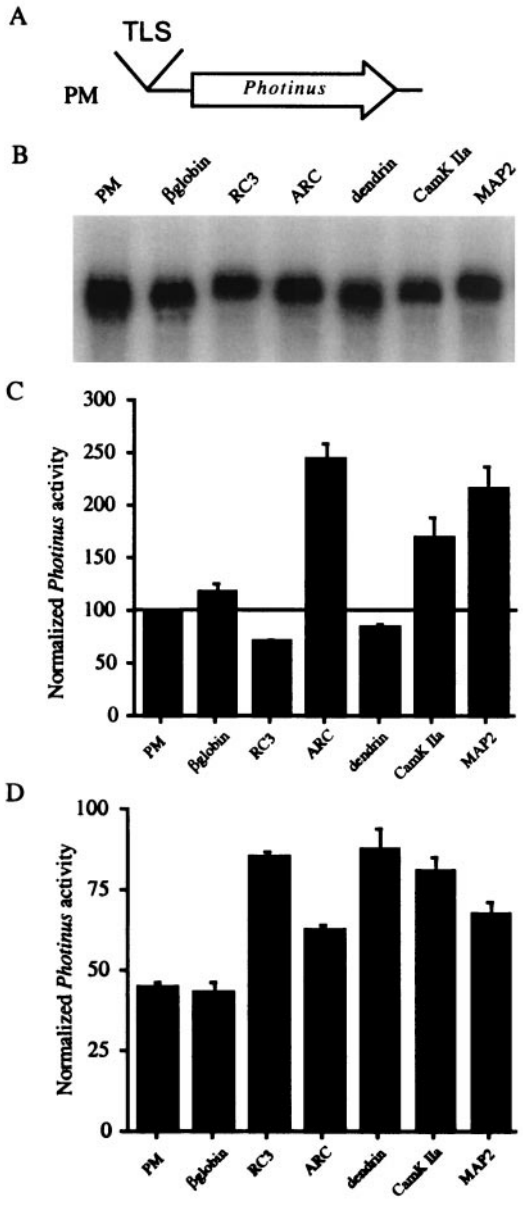

Fig. 1. Analysis of monocistronic mRNAs containing the transcribed leader sequences of dendritically localized mRNAs. (*A*) Schematic illustration of monocistronic reporter mRNAs containing the transcribed leader sequences (TLS) 5′ of the *Photinus* luciferase gene. (*B*) Northern blot analysis of monocistronic mRNAs using equivalent amounts of total RNA from B104 cells transfected with the monocistronic constructs indicated and probed with a radiolabeled cRNA to the *Photinus* luciferase gene. (*C*) *Photinus* luciferase activity generated by monocistronic reporter mRNAs. Luciferase activity was normalized to that generated by the parent vector *P*M. (*D*) Monocistronic constructs were cotransfected with either a plasmid expressing hypophosphorylated 4E-BP1 or control plasmid and assayed for luciferase activity. The activities obtained in cells cotransfected with the mutated 4E-BP1 is represented as a percentage of the activity obtained in cells cotransfected with the control plasmid.

intensity of the autoradiographic bands between monocistronic constructs (Fig. 1*B*), indicating that the leader sequences do not affect the transcription or the stability of the monocistronic mRNAs. The *Photinus* luciferase activities from each construct were assayed (Fig. 1*C*); results were normalized for transfection efficiency by using CAT activity, which was coexpressed from the plasmid. The translation efficiency of mRNAs containing the ARC, CamK IIa, and MAP2 leader sequences was enhanced up to 2.5-fold as compared with the *P*M or to the β-globin/*P*M mRNA, both of which had similar translation efficiencies. The translation efficiency of monocistronic mRNAs containing either the RC3 or dendrin leader sequences was approximately 20% lower than the *P*M or β-globin/*P*M mRNAs.

To determine whether these transcribed leader sequences initiated translation by a cap-dependent and/or cap-independent mechanism, a hypophosphorylated form of 4E-BP1 (see *Materials and Methods*) was used to block cap-dependent translation by binding to the cap-binding protein eIF4E (19). The monocistronic constructs were cotransfected with a construct expressing the mutated 4E-BP1 into B104 cells, and the results were normalized to the activities obtained when the monocistronic constructs were cotransfected with a control plasmid, expressed as 100% (Fig. 1*D*). The mutated 4E-BP1 reduced translation of all constructs. Both the *P*M and the β-globin/*P*M mRNAs were reduced by 55% and 57%, respectively, but the translation of the mRNAs with RC3, ARC, dendrin, CamK IIa, and MAP2 leader sequences was much less reduced. Translation of the ARC/*P*M and MAP2/*P*M mRNAs was reduced by 38% and 32%, respectively, whereas that of the RC3/*P*M and CamK IIa/*P*M was reduced by ≈20%, and that of the dendrin/*P*M was reduced by only 13%. These differences are statistically significant (see *Materials and Methods*) and are consistent with results obtained when a hairpin structure was inserted at the 5′ end of these leader sequences to inhibit ribosome scanning (data not shown). Taken together, these results suggest that translation of monocistronic mRNAs containing the RC3, ARC, dendrin, CamK IIa, and MAP2 leader sequences can occur by both cap-dependent and cap-independent mechanisms.

5′ Leader Sequences of Five Dendritically Localized mRNAs Contain IRESes. To determine whether cap-independent translation occurred via internal initiation, the leader sequences of ARC, dendrin, CamK IIa, and MAP2 were tested in the intercistronic region of a dual luciferase dicistronic mRNA (*RP*, Fig. 2*A*). Dicistronic mRNAs were expressed in transfected neural cell lines B104, N2a, and C6 (Fig. 2*B*). The negative control β-globin/*RP* mRNA, which contains the β-globin 5′ UTR in the intercistronic region of *RP*, yielded a *Photinus*/*Renilla* luciferase activity ratio (*P*:*R*) that was similar to that of *RP* mRNA. In contrast, the positive control EMCV/*RP* mRNA, which contains the encephalomyocarditis virus (EMCV) IRES in the intercistronic region, yielded a *P*:*R* ratio of 6-fold to 7-fold that of *RP* mRNA (Fig. 2*B*). The dicistronic mRNAs that contained the 5′ leader sequences of the dendritically localized mRNAs also yielded enhanced *P*:*R* ratios. The ARC leader sequence enhanced *Photinus* luciferase activity the most, with a *P*:*R* ratio of up to 13-fold that of *RP*, 2-fold greater than that obtained with the EMCV IRES. The *P*:*R* ratios obtained with the different leader sequences were similar in all three neural cell lines. Moreover, when tested in the nonneural cell lines NIH 3T3 and LMTK$^-$, the *P*:*R* ratios were similar to or higher than those obtained in the neural cell lines, indicating that the activity of these IRESes was not restricted to neural cells (Fig. 2*C*).

To exclude the possibility that the increased *P*:*R* ratios were caused by the generation of monocistronic *Photinus* mRNAs by splicing or by promoter activity, RNA was isolated from N2a cells that were transfected with the different dicistronic constructs and analyzed on Northern blots (Fig. 2*D*) by using a

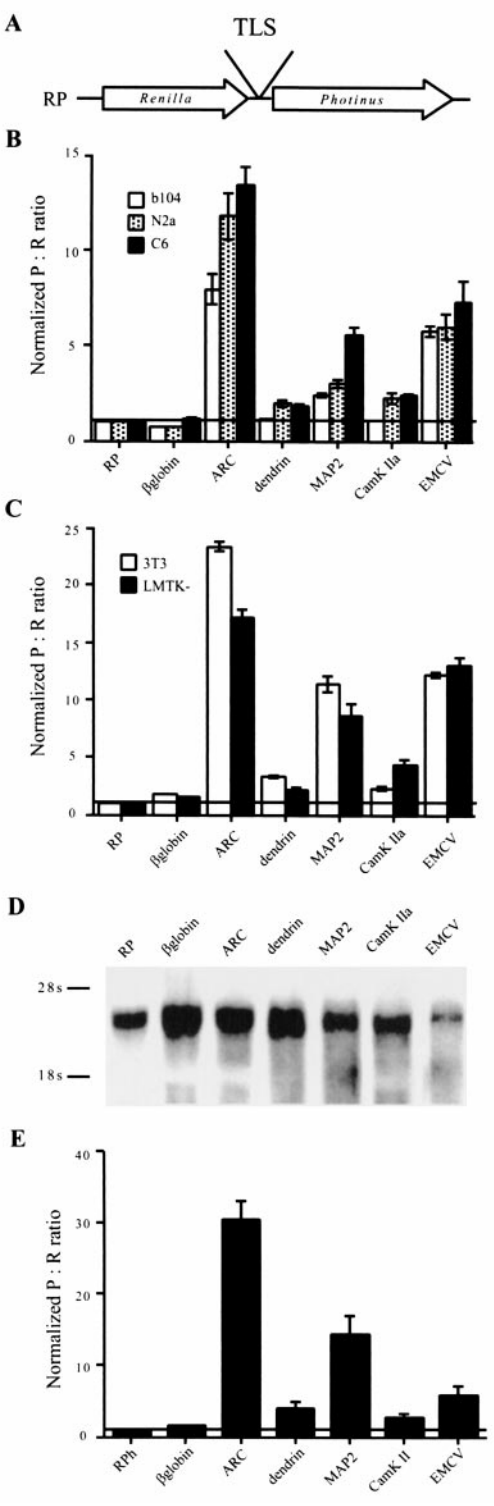

Fig. 2. Analysis of dicistronic mRNAs containing the transcribed leader sequences of dendritically localized mRNAs. (*A*) Schematic representation of the dicistronic luciferase mRNAs. IRES activity generated by each construct after transfection into neural (*B*) and nonneural (*C*) cell lines is shown as the ratio of *Photinus* to *Renilla* luciferase activities (*P*:*R*) and normalized to the activity generated by the parent construct *RP*. (*D*) Total RNA harvested from B104 cells transfected with the dicistronic constructs was hybridized with a cRNA probe specific for the ORF of the *Photinus* luciferase gene. The migration of the 28S and 18S rRNAs is indicated. (*E*) Analysis of luciferase activity generated from each dicistronic construct in the presence of the hairpin structure. Luciferase activity is shown as the *P*:*R* ratio and is normalized to the activity of the hairpin control construct, *RP*h.

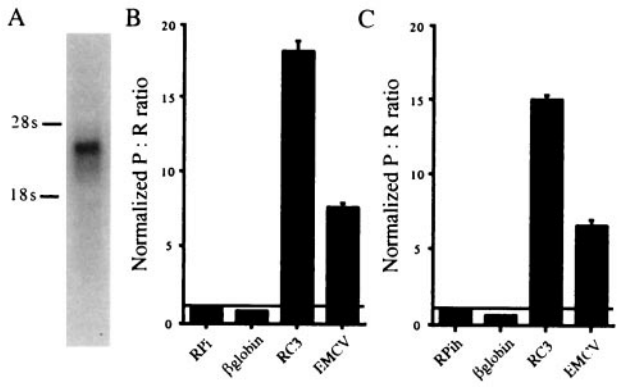

Fig. 3. Analysis of the RC3 IRES in the intronless *RP*i construct. (*A*) Northern blot analysis of total RNA extracted from B104 cells transfected with the RC3/*RP*i construct containing the RC3 leader sequence and hybridized with a cRNA probe to the *Photinus* luciferase gene. (*B*) *P*:*R* ratios produced from dicistronic luciferase mRNAs containing the β-globin, RC3, and EMCV leader sequences and normalized to the *P*:*R* ratio generated by *RP*i. (*C*) *P*:*R* ratios in the presence of a 5′ hairpin structure after transfection into B104 cells.

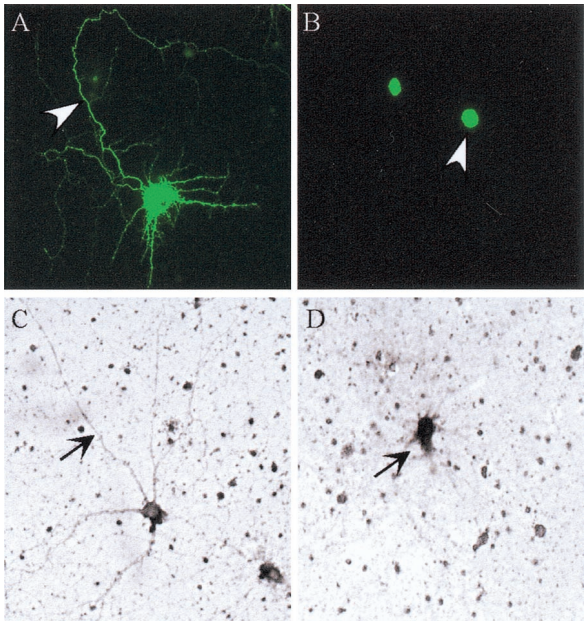

Fig. 4. Analysis of the RC3 3′ UTR as a dendritic targeting sequence and demonstration of translation in dendrites. Cultured hippocampal neurons were transfected with a construct encoding EYFP with a nuclear-localization sequence containing either the RC3 3′ UTR (*A*) or a simian virus 40 polyadenylation sequence alone (*B*). *In situ* hybridization of neurons transfected with the EYFP/NLS constructs with (*C*) or without (*D*) the RC3 3′ UTR using a digoxygenin-labeled cRNA probe to the EYFP coding region. Arrows in *A* and *C* indicate the localization of the EYFP protein and exogenous mRNA, respectively, in neuronal processes of transfected neurons. Arrows in *B* and *D* identify the nucleus of a transfected neuron.

cRNA probe to the *Photinus* gene. The results indicate that the dicistronic mRNAs in this analysis were intact, and that the presence of the leader sequences did not result in the appearance of additional, shorter bands.

To address the possibility that the increased *P*:*R* ratios occurred by reinitiation from the first cistron or leaky scanning, the leader sequences were cloned into a vector (*RP*h) that contained an inverted repeat sequence 5′ to the first cistron. The resulting hairpin blocks scanning ribosomes and impedes the cap-dependent translation of the first cistron, but it should not affect translation of the second cistron by internal initiation. These constructs were transfected into B104 cells and assayed for luciferase activity. The *P*:*R* ratios for all four leader sequences and the EMCV IRES were significantly above background *RP*h ratios (Fig. 2*E*), lending further support to the argument that the activity of these leader sequences did not depend on the translation of the first cistron. The *P*:*R* ratio obtained with a construct containing the β-globin 5′ leader was not significantly different from that obtained with the *RP*h construct. These results indicate that the leader sequences of the four dendritically localized mRNAs contain IRESes.

The 5′ UTR of RC3 was also analyzed in the dual luciferase dicistronic mRNA. Northern analysis of the RC3 5′ UTR in the *RP* construct resulted in the appearance of two mRNAs (data not shown). Removal of the chimeric intron from the *RP* vector resulted in the production of one mRNA of the correct size predicted for the dicistronic mRNA (Fig. 3*A*). The leader sequence of β-globin mRNA as well as the EMCV IRES was cloned into the intercistronic region of this modified intronless construct (*RP*i) and was analyzed in transiently transfected B104 cells. The resulting β-globin and EMCV *P*:*R* ratios were similar to those generated by the dicistronic mRNAs containing the chimeric intron (compare Figs. 2*B* and 3*B*). The *P*:*R* ratios produced from dicistronic mRNAs containing the RC3 5′ UTR were approximately 18-fold higher than *RP*i and approximately 3-fold higher than that obtained with the EMCV IRES. The *P*:*R* ratio obtained with constructs containing a hairpin structure 5′ of the first cistron was not significantly different from those obtained with the *RP* construct (compare Fig. 3 *B* and *C*). These findings indicate that the RC3 leader sequence also contains an IRES.

Analysis of the RC3 IRES in Primary Hippocampal Neurons. To analyze protein synthesis in dendrites, a monocistronic fluorophore-reporter construct was engineered to target the mRNAs to dendritic processes. Targeting sequences have been identified within the 3′ UTRs of several dendritically localized mRNAs (24). To determine whether the RC3 3′ UTR functioned similarly, it was included in the 3′ UTR of a monocistronic mRNA-encoding EYFP along with an NLS (EYFP/NLS; see *Materials and Methods*). The inclusion of the RC3 3′ UTR resulted in the expression of a significant amount of the fluorescent protein in dendrites of transfected neurons (Fig. 4*A*); constructs lacking this 3′ UTR expressed the fluorescent protein only in the nucleus (Fig. 4*B*). This finding suggested that the RC3 3′UTR could target mRNA to the dendrites of transfected neurons. *In situ* hybridization confirmed that reporter mRNAs containing the RC3 3′ UTR were localized throughout the processes of transfected hippocampal neurons (Fig. 4*C*), whereas reporter mRNAs lacking this 3′ UTR were confined to the cell body (Fig. 4*D*). In addition, colocalization studies using the MAP2 antibody, which specifically stains dendrites, showed that mRNAs containing the RC3 3′ UTR occurred within dendrites (data not shown). These studies validated the use of the RC3 3′ UTR in directing mRNAs to the dendritic compartment, and also demonstrated that translation occurs in dendrites.

To examine whether IRES-mediated translation can occur within dendrites, we used a dicistronic mRNA that contained ECFP as the first cistron and EYFP as the second cistron, and also contained the RC3 3′ UTR. To limit diffusion from the site of synthesis and facilitate quantification of *de novo* local protein synthesis, both fluorescent proteins were engineered to contain a destabilizing motif (see *Materials and Methods*), which shortened the half-lives of these proteins to approximately 4 h. In an initial analysis of the dicistronic fluorophore mRNAs, the constructs were tested in transfected N2a cells. IRES activities using this construct were similar to those observed using the dual luciferase vector (data not shown).

Hippocampal neurons were transfected with dicistronic ECFP/EYFP constructs containing the 5′ leader sequences from β-globin and RC3 mRNAs within the intercistronic region. Fluorescence measurements were taken from the cell body as well as from the dendrites. Fluorescent overlay images using ECFP and EYFP filters were taken from the dendritic fields of two neurons transfected with dicistronic fluorophore constructs containing either the β globin leader sequence (Fig. 5*A*) or RC3 leader sequence (Fig. 5*B*) in the intercistronic region, respectively. Transfection of hippocampal neurons with the dicistronic construct containing the β-globin 5′ UTR resulted in mainly cap-dependent ECFP expression (visualized as cyan). However, the mRNA containing the RC3 leader sequence demonstrated both cap-dependent translation and IRES-dependent translation (visualized as yellow) generating turquoise. Fluorescence intensity of both cistrons was quantified from images and presented as a yellow:cyan ratio, as shown in Fig. 5*C*. As can be seen, the RC3 leader functions as an IRES in both the cell body and the dendrites. Distribution of the activity of the RC3 IRES appeared to be punctate in the dendrites, which may indicate increased activity near synapses. In addition, the relative activity of the RC3 IRES was approximately 2-fold more active in the dendrites than in the cell body. A Northern blot of these dicistronic fluorophore mRNAs contained a single hybridizing band of the correct molecular weight (data not shown).

Discussion

Inasmuch as many synaptic modifications do not persist when translation is inhibited (25), translation has been suggested to have an essential role in mediating changes in the morphology and efficacy of individual synapses in response to synaptic activity (26). Moreover, it is thought that some synaptic changes result from translation at postsynaptic sites rather than from cell-wide changes in protein synthesis (6, 7). Such local translation would enable different synapses to be modified independently within the same cell. Evidence to support the notion of local protein synthesis includes the observations that components of the translation machinery occur in dendrites (3–5), that protein synthesis can occur at these cellular processes (9, 27, 28), and that many dendritically localized mRNAs encode proteins that are integral to synaptic modification, including structural proteins (ARC, MAP2), growth factors, ionotropic receptors, and kinases (CaMK IIa; reviewed in ref. 1). Taken together, these observations are consistent with the notion that translation in dendrites may be used to rapidly and locally synthesize some of the proteins required to strengthen active synapses.

In the present study, we found that the leader sequences from five dendritically localized mRNAs contain IRESes that mediate cap-independent translation. We also observed that, for RC3 within dendrites of cultured hippocampal neurons, translation occurred by both cap-dependent and cap-independent mechanisms. Furthermore, translation by the cap-independent mechanism was relatively more efficient in the dendrite than in the cell body.

The IRESes identified in these studies may serve to ensure or regulate translation of specific messages under varying conditions and may be important for alterations in synaptic plasticity. Our identification of an IRES in the 5′ UTR of the mRNA encoding the α-subunit of Cam Kinase II is consistent with the results of a recent study showing increased translation of this mRNA after treatment of developing synapses with *N*-methyl-D-aspartate receptor agonists, even though total protein synthesis was impaired (13). It has also been shown recently that the 5′ UTR of the mRNA encoding Pim kinase, a protein that has recently been implicated in the stabilization of long-term potentiation (29), also contains an IRES (30).

Inasmuch as ribosomes and initiation factors are present at low concentrations in dendrites, IRESes may be used by dendritically localized mRNAs to increase the ability to recruit translation machinery within this cellular compartment. Indeed, IRESes appear to be used by some mRNAs when cap-dependent translation is reduced or blocked (31–33). For instance, overall levels of translation are reduced during apoptosis and during various types

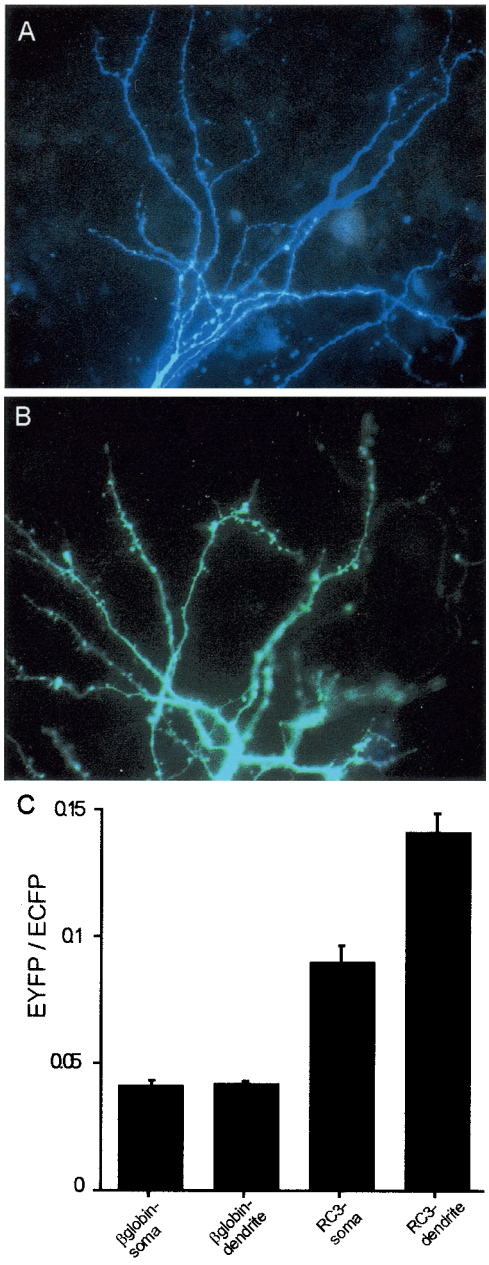

Fig. 5. *In situ* analysis of IRES activity in hippocampal neurons. Dicistronic constructs were created containing ECFP and EYFP as the first and second cistron, respectively. The 5′ leader sequence from β-globin or RC3 mRNAs was inserted into the intercistronic region. The constructs also contained the RC3 3′ UTR to direct transport of the mRNA into the dendrites. The dicistronic constructs were transfected into mature primary hippocampal neurons that were cultured *in vitro* for 3 weeks. Fluorescent photomicrographs of dendrites transfected with the dicistronic construct containing the β-globin (*A*) or RC3 (*B*) leader sequence in the intercistronic region under both ECFP and EYFP filters are shown. Cap-dependent translation is seen as blue; IRES-dependent translation is seen as yellow. Where both cap- and IRES-dependent translation occur, the blue and yellow fluorescence combine to exhibit turquoise. (*C*) Fluorescent emission was measured from each cistron in the cell body and in the dendrites. IRES activity is quantified as a ratio of the fluorescence emitted from the second cistron (EYFP) to the fluorescence emitted from the first cistron (ECFP).

of cellular stress such as ischemia (15); in these situations, IRES-mediated translation continues (32–34). In addition, several reports have indicated that synaptic activity leads to an increase in protein synthesis in or near the active synapses (9, 27), an increase which could saturate the cap-dependent translation machinery in dendrites. Nonetheless, it remains to be determined whether internal initiation provides a way to initiate translation of dendritically localized messages in response to synaptic stimuli.

The present findings add five additional members to the relatively short list of cellular IRESes described to date. It is notable that these mRNAs are spatially localized within the neuron. Analyzing the relationship of the translation of such mRNAs to neuronal plasticity may uncover alternative mechanisms by which dendritic translation affects synaptic efficacy.

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