Mammalian Target of Rapamycin (mTOR) Tagging Promotes Dendritic Branch Variability through the Capture of Ca\(^2\+)/Calmodulin-dependent Protein Kinase II \(\alpha\) (CaMKII\(\alpha\)) mRNAs by the RNA-binding Protein HuD*

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Background: Memory requires protein synthesis of dendritic CaMKII\(\alpha\).

Results: HuD directs CaMKII\(\alpha\) expression in a branch-specific manner. mTOR inhibition reduces HuD binding and promotes deadenylation of CaMKII\(\alpha\) mRNA.

Conclusion: mTOR activity tags synapses, allowing HuD to capture CaMKII\(\alpha\) in a branch-specific manner.

Significance: mTOR and HuD provide a molecular model for the synaptic tagging and capture hypothesis.

The fate of a memory, whether stored or forgotten, is determined by the ability of an active or tagged synapse to undergo changes in synaptic efficacy requiring protein synthesis of plasticity-related proteins. A synapse can be tagged, but without the “capture” of plasticity-related proteins, it will not undergo long lasting forms of plasticity (synaptic tagging and capture hypothesis). What the “tag” is and how plasticity-related proteins are captured at tagged synapses are unknown. Ca\(^2\+)/calmodulin-dependent protein kinase II \(\alpha\) (CaMKII\(\alpha\)) is critical in learning and memory and is synthesized locally in neuronal dendrites. The mechanistic (mammalian) target of rapamycin (mTOR) is a protein kinase that increases CaMKII\(\alpha\) and mRNA by shortening its poly(A) tail. Overexpression of the RNA-stabilizing protein HuD increased CaMKII and mRNA expression in dendrites may yield information regarding the importance of dendritic branches in memory formation.

Activation of mTOR kinase is required for protein synthesis-dependent, late phase long term potentiation (LTP) and memory consolidation (1, 2). mTOR consists of two subunits, mTORC1 and mTORC2. mTORC1, a serine/threonine kinase, promotes cap-dependent translation by phosphorylating p70 S6 kinase and elf4E-binding protein (3). One notable mRNA whose translation is regulated by mTORC1 is Ca\(^2\+)/calmodulin-dependent protein kinase II \(\alpha\) (CaMKII\(\alpha\)) (4, 5). CaMKII\(\alpha\) is important for the induction and maintenance of LTP and memory (6). The importance of locally translated CaMKII\(\alpha\) mRNA in memory consolidation was demonstrated in a mouse where the dendritic targeting sequence of CaMKII\(\alpha\) in the genome was disrupted (7). Moreover, synapses that express protein synthesis-dependent LTP tend to occur on one dendritic daughter branch as opposed to the synapses of both daughter branches (8). Thus, further insight into the subcellular loci of CaMKII\(\alpha\) expression in dendrites may yield information regarding the importance of dendritic branches in memory formation.

The expression of the RNA-binding protein HuD is correlated with both spatial learning and contextual fear conditioning.

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4 The abbreviations used are: mTOR, mechanistic (mammalian) target of rapamycin; CaMKII\(\alpha\), Ca\(^2\+)/calmodulin-dependent protein kinase II \(\alpha\); RRM, RNA recognition motif; LTP, long term potentiation; mTORC, mTOR complex; eGFP, enhanced GFP; DIV, days in vitro; Rapa, rapamycin; APS, (2R)-amino-5-phosphonovaleric acid; DMSO, dimethyl sulfoxide; P-S6, phospho-S6; FISH, fluorescence in situ hybridization; BVI, branch variability index; myr-dGFP, myristoylated destabilized GFP; NMDAR, NMDA receptor; KD, knockdown; ANOVA, analysis of variance.
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(9–11). Furthermore, expression of several HuD target mRNAs is associated with improved cognition (9, 12, 13). Recently, we determined that mTORC1 kinase serves as a switch for translation of specific mRNAs such as CaMKIIα through HuD. We demonstrated that when mTORC1 is active HuD binds to its high affinity target mRNAs including CaMKIIα, stabilizing the mRNA and promoting its translation. When mTORC1 is inhibited, CaMKIIα mRNA is degraded, thus releasing HuD and allowing it to bind to low affinity target mRNAs such as the voltage-gated potassium channel Kv1.1 (5). How CaMKIIα mRNA is degraded is unclear. Collectively, these data strongly support a role for mTOR activity and HuD to promote the translation of mRNAs that support learning and memory.

The synaptic tagging and capture hypothesis proposes that the synapses activated during early LTP become tagged in a protein synthesis-independent manner (14). For the tagged synapse to undergo lasting changes in synaptic efficacy, it must capture plasticity-related proteins or the mRNAs that code for these proteins (15). The requirement for protein synthesis comes from studies that demonstrate that the conversion of early LTP to late phase LTP is blocked with the addition of protein synthesis inhibitors such as the mTORC1 inhibitor rapamycin (16, 17). Although a great deal is known about global protein synthesis inhibitors such as the mTORC1 inhibitor rapamycin (1,200; Sigma, rabbit anti-ocy-myc (1,200, Sigma), rabbit anti-phospho-S6 (P-S6) (1,50; Cell Signaling Technology), and chicken anti-MAP2 (1,200; Abcam). Secondary antibodies from Life Technologies used at 1:400 were Alexa Fluor 488 anti-chicken, Alexa Fluor 555 anti-mouse, and Alexa Fluor 647 anti-rabbit.

Fluorescence in Situ Hybridization (FISH)—For CaMKIIα or HuD mRNA detection, fluorescence in situ hybridization was conducted using the ViewRNA ISH Cell Assay kit (Affymetrix) as described in Cajigas et al. (19). The CaMKIIα and HuD probe sets were designed commercially by Affymetrix. Briefly, primary hippocampal neurons (DIV 20–21) were fixed at room temperature for 30 min with a 4% paraformaldehyde solution (4% paraformaldehyde, 5,4-glucose, 0.01M sodium metaperio-
date in lysine-phosphate buffer). Proteinase K treatment was omitted, and the rest of the hybridization was completed according to the manufacturer’s instructions. The cells were then washed with PBS and blocked with 4% goat serum in PBS for 1 h followed by incubation in primary antibody (chicken anti-MAP2 or chicken anti-GFP) overnight at 4 °C. After three washes with PBS, the cells were incubated with the appropriate secondary antibody for 1 h at room temperature and washed with PBS. The coverslips were then mounted with an antifading mounting medium and imaged as described above.

Quantification of Phospho-S6 Puncta and CaMKIIα mRNA Puncta—Images were acquired using a Leica SP5 confocal microscope (63× objective lens; numerical aperture, 1.2) with sequential scanning. Series of z-stacks were collected at 0.5-μm intervals for a total of 5.0 μm. Dendrites were chosen blindly based on MAP2 or eGFP signal. Following image acquisition, a binary mask of equally thresholded images was created using Meta Imaging Series 7.8. To measure branch variability, 10-μm-long regions of interest were drawn before and after the primary branch point of the MAP2 or eGFP dendrite as described by Govindarajan et al. (8). P-S6 punctum intensity in the primary and secondary branches and CaMKIIα punctum intensity in the cell body and primary and secondary branches were measured using integrated morphometry image analysis. P-S6 intensity in the cell body was taken as a ratio over eGFP or MAP2 as the signal was not punctate in the cell body. Individual puncta were counted in the primary and secondary branches similar to Cajigas et al. (19). To determine whether mTOR was equally or differentially active between daughter branches, the number of P-S6 puncta/10-μm area after the branch point was determined for each daughter branch emerging from a single parent dendrite. The absolute value of the difference in punc-
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Results

NMDAR Signaling Activates mTORC1 in Cultured Hippocampal Neurons—For synapses to be tagged, they must be stimulated by synaptic activity, usually requiring N-methyl-d-aspartate (NMDA) receptor activation. Using a simplified system, we have shown previously that NMDAR activity promotes the phosphorylation of mTORC1, and acute rapamycin treatment reduces it in cultured hippocampal and cortical neurons (21–28 DIV) (25). However, the subcellular localization of mTORC1 activity has not been determined. To this end, we treated neurons with vehicle, AP5, or the mTORC1 inhibitor rapamycin and stained for the downstream marker of active mTORC1, P-S6, a key ribosomal protein. Thus, we predict that during elevated spontaneous NMDAR activity, such that occurs in dissociated neurons after several weeks in culture (14), mTORC1 will be active throughout the dendritic arbor aspartate (NMDA) receptor activation. Using a simplified system, we have shown previously that NMDAR activity promotes the phosphorylation of mTORC1, and acute rapamycin treatment reduces it in cultured hippocampal and cortical neurons (21–28 DIV) (25). However, the subcellular localization of mTORC1 activity has not been determined. To this end, we treated neurons with vehicle, AP5, or the mTORC1 inhibitor rapamycin and stained for the downstream marker of active mTORC1, P-S6, a key ribosomal protein. Thus, we predict that during elevated spontaneous NMDAR activity, such that occurs in dissociated neurons after several weeks in culture (14), mTORC1 will be active throughout the dendritic arbor (Fig. 1A). As expected, mTORC1 was strongly activated in the cell body (Fig. 1B) and primary and secondary branches (Fig. 1, C and D) of cultured primary hippocampal neurons as indicated by the change in signal intensity of P-S6 puncta or hot spots with rapamycin. To determine whether mTORC1 activity is equally or differentially stimulated between two daughter branches that emerge from a single parent dendrite, we counted the number of P-S6 hot spots/10-μm area directly after the branch point similar to Govindarajan et al. (8). We arbitrarily assigned one daughter branch A and the other B (Fig. 1A, schematic, yellow and white boxes). We then took the absolute value of the difference in P-S6 hot spots between branches A and B. As predicted, there was relatively little difference between the two daughter branches, only differing in P-S6 punctum number by ∼1 hot spot when mTORC1 was active (branch with most puncta averaged ∼3 ± 0.24 versus the branch with the fewest number of puncta averaging ∼2 ± 0.41). Notably, the signal intensity of the P-S6 hot spots was significantly reduced with mTORC1 inhibition; however, the remaining signal between branches was relatively the same (Fig. 1E; BVI for DMSO,
FIGURE 1. NMDAR signaling leads to mTORC1 activity throughout the dendritic arbor. A, schematic of neuron (top) shows where punctum signal intensity measurements were taken for quantitative analysis throughout all figures. Boxed regions (primary branch, green; daughter branch A, yellow; daughter branch B, white) represent 10-μm segments prior to and after the branch point used for analysis. Immunostaining of P-S6 hot spots in DMSO (carrier)- and rapamycin (200 nM)-treated cultured hippocampal neurons. Cell body images were taken with a lower gain relative to dendrites to avoid saturation of signal (left). MAP2 expression (not shown) was used to outline the dendrites of the representative neurons. Colored arrows refer to the corresponding blown up region of the dendrite that is outlined with the dotted line in the same color to the right. Images were pseudocolored, and three-dimensional rendering was achieved using the Interactive 3D Surface Plot plug-in in ImageJ to demonstrate differences in signal intensity. Scale bar, 10 μm. B, P-S6 punctum signal intensity was measured in the cell body and normalized by area for DMSO- and rapamycin-treated neurons. Note that P-S6 punctum intensity decreases with rapamycin treatment in the cell body. *, p < 0.05 by unpaired Student’s t test. DMSO, n = 11 neurons; Rapa, n = 12 neurons. C, P-S6 punctum signal intensity was measured in a region 10 μm before the primary branch point of DMSO- and rapamycin-treated neurons. DMSO, n = 13 primary dendrites; Rapa, n = 17 primary dendrites. D, P-S6 punctum signal intensity was measured in a region 10 μm after the primary branch point of DMSO- and rapamycin-treated neurons. *, p < 0.05 by unpaired Student’s t test. DMSO, n = 26 secondary branches; Rapa, n = 24 secondary branches. E, change in mTORC1 activity between daughter branches was determined by counting the number of P-S6 puncta in each daughter branch/10-μm area after each branch point and using the following equation: ΔP-S6 hot spots = [Daughter branch A/Area] – [Daughter branch B]. DMSO, n = 16 daughter branch pairs; Rapa, n = 14 daughter branch pairs. F–J, cultured hippocampal neurons were treated with vehicle (H2O) or AP5 (100 μM) and immunostained for P-S6 puncta. MAP2 (not shown) expression was used to outline the dendrites of the representative neurons. Quantification was performed on non-saturated images of cell bodies. Quantification of P-S6 puncta was performed as described above for the rapamycin treatment. Images were pseudocolored, and three-dimensional rendering was achieved using the Interactive 3D Surface Plot plug-in in ImageJ. * in y axis in H indicates multiplication. Scale bar, 10 μm. Vehicle, n = 19 cell bodies, 19 primary (1°) branches, 39 secondary (2°) branches, and 20 paired daughter branches; AP5, n = 16 cell bodies, 18 primary branches, 31 secondary branches, and 17 paired daughter branches. **, p < 0.01 by unpaired Student’s t test. Error bars represent S.E.
0.94 ± 0.30; BVI for Rapa, 0.71 ± 0.35; single t test not significantly different from zero).

In contrast to rapamycin, blocking NMDAR signaling with AP5 had a smaller effect of ~30% versus a ~60% reduction of the P-S6 signal in the cell body (Fig. 1, A and B and F and G). Interestingly, AP5 significantly reduced the signal intensity of P-S6 in the primary, parent dendrite by ~90% (Fig. 1H; vehicle (water), 1 ± 0.27; AP5, 0.10 ± 0.03) and the secondary branch by ~79% (Fig. 1, F and I; vehicle (water), 1.00 ± 0.17; AP5, 0.21 ± 0.05). Similar to rapamycin, the number of detectable puncta did not change between daughter branches (Fig. 1, E and J). These data suggest that NMDAR activity stimulates mTORC1 throughout the dendritic arbor and can be specifically blocked with either AP5 or rapamycin treatment.

NMDAR and mTORC1 Activity Is Required for CaMKIIα Branch-specific Expression—NMDAR activation stimulates the mTOR-dependent, local protein synthesis of CaMKIIα mRNA (4). However, it is unknown whether mTORC1 differentially regulates CaMKIIα protein expression in one daughter branch over the other. To answer this question, we determined whether CaMKIIα protein expression was branch-specific using immunofluorescence with blockers of NMDAR/mTORC1 activity. eGFP expression allowed us to clearly visualize individual neurons and normalize signal by volume. Although the cell body CaMKIIα signal in neurons was highly variable, NMDAR inhibition with AP5 but not mTORC1 inhibition with rapamycin reduced CaMKIIα protein expression dramatically by ~66% (Fig. 2, A and B and F and G). These results suggest that CaMKIIα expression in the cell body may be independent of mTORC1 activity.

Next, we measured CaMKIIα in the dendrites by determining the average signal intensity in the primary apical dendrite prior to the first branch and normalized by eGFP as a volume control (Fig. 2, A, C, F, and H). Inhibition of mTORC1 by rapamycin (Fig. 2, A and C) or NMDARs by AP5 (Fig. 2, F and H) reduced the dendritic expression of CaMKIIα in the primary parent branch by ~50 and 40%, respectively (Fig. 2C: DMSO, 1.00 ± 0.22; Rapa, 0.49 ± 0.05; Fig. 2G: vehicle, 1.00 ± 0.15; AP5, 0.60 ± 0.10). Next, we measured the average signal intensity of CaMKIIα in each daughter branch 10 μm from the branch point normalized by eGFP. Again, reducing mTORC1 activity either by NMDAR antagonism (AP5) or rapamycin decreased the overall expression of CaMKIIα in the secondary branches (Fig. 2, A, D, F, and J). To determine whether CaMKIIα was differentially expressed between daughter branches, we determined its BVI by measuring the average signal intensity in the primary apical dendrite minus daughter branch A minus daughter branch B. This difference was divided by the average BVI for control neurons. In this case, a value of 0 indicates that the protein is equally distributed between daughter branches (see “Experimental Procedures” for the equation). As the BVI moves away from 0, protein expression becomes more polarized in one daughter branch over the other. Indeed, CaMKIIα protein was enriched in one daughter branch by at least ~2-fold when mTORC1 was active relative to neurons treated with AP5 or the mTORC1 inhibitor rapamycin (Fig. 2E: BVI for DMSO, 1.00 ± 0.06; BVI for Rapa, 0.45 ± 0.07; Fig. 2F: BVI for vehicle, 1.00 ± 0.27; BVI for AP5, 0.24 ± 0.09).

Branch-specific Expression of Kv1.1 Does Not Require mTORC1 Activity—To determine whether branch-specific expression is generally dependent on mTORC1, we examined the expression of Kv1.1 protein whose dendritic expression is negatively regulated by mTORC1 activity. As observed previously (5, 25, 26), mTORC1 inhibition increased Kv1.1 protein ~100% in the primary and ~70% in the secondary dendrites (primary dendrite: DMSO, 1.00 ± 0.17; Rapa, 2.04 ± 0.45; secondary dendrite: DMSO, 1.00 ± 0.13; Rapa, 1.72 ± 0.27; Fig. 3, A–D). Although we did not observe changes in BVI when mTORC1 activity was disrupted, the BVI of ~1 suggests that Kv1.1 protein expression was more abundant in one daughter branch over the other under both conditions (Fig. 3E). These results altogether suggest that both CaMKIIα and Kv1.1 expression is branch-specific, favoring one daughter branch over the other in a single neuron. In contrast, mTORC1 activity regulates the drop in the branch-specific expression of CaMKIIα but not of Kv1.1.

CaMKIIα mRNA Targets One Daughter Branch over the Other in a Single Neuron When mTORC1 Is Active—An unresolved debate concerning the synaptic tagging and capture hypothesis is whether it is the mRNA or protein that is “captured” in a site-specific manner. Although CaMKIIα protein is branch-specific (Fig. 2), it is unclear whether the mRNA is as well. To answer this question, we performed FISH against CaMKIIα mRNA when mTORC1 was active or inhibited with rapamycin. Control and rapamycin-treated neurons were probed for CaMKIIα mRNA and quantified (Fig. 4A). As a negative control, we used a sense probe that did not detect any signal (Fig. 4B). Consistent with CaMKIIα protein levels, there was no change in the number of mRNA granules in the cell body when normalized by area (Fig. 4, A and C). In contrast, there was a significant reduction in total CaMKIIα mRNA granules in the primary and secondary dendritic branches of rapamycin-treated neurons (Fig. 4, D and E).

To determine whether the mRNA targets one branch over the other, we determined whether there were more CaMKIIα mRNA-positive granules in one daughter branch over the other in a single neuron. As expected, we detected a range between ~4 (high branch) and ~1 (low branch), with ~3 more granules per 10-μm segment that were selectively targeted to one branch over the other (Fig. 4F) when mTORC1 was active (Fig. 4F; DMSO, 2.5 ± 0.28; Rapa, 0.65 ± 0.15). As seen with the protein, the number of mRNA-positive granules was decreased with rapamycin, reducing the branch-selective expression of CaMKIIα mRNA. Of note, the signal intensity of the granules that remained present after rapamycin treatment is relatively equal to the signal intensity of those that were present when mTORC1 was active (Fig. 4G). Consistent with the branch-selective expression of CaMKIIα protein, these results suggest that when mTORC1 is active CaMKIIα mRNA localizes to one daughter branch over the other within a single neuron.

mTORC1 Inhibition Results in the Rapid Degradation of CaMKIIα mRNA by Shortening of the Poly(A) Tail—In light of these data, two questions remain unanswered: 1) what mediates
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Figure 2. NMDAR and mTORC1 activity lead to branch-specific expression of CaMKIIα protein levels that is reduced with NMDAR/mTORC1 blockade. A, eGFP-expressing cultured hippocampal neurons were treated with DMSO or rapamycin and immunostained for CaMKIIα. Representative neurons are shown. Colored arrows refer to the corresponding blow-up region of the dendrite that is outlined with the dotted line in the same color to the right. CaMKIIα images were pseudocolored, and three-dimensional rendering was achieved using the Interactive 3D Surface Plot plug-in in ImageJ to demonstrate differences in signal intensity. Scale bar, 10 μm. B, the signal intensity of CaMKIIα in the cell body of neurons treated with DMSO or rapamycin was measured as a ratio over eGFP. Only unsaturated cell bodies were used for the quantification. DMSO, n = 18 neuronal cell bodies; Rapa, n = 13 neuronal cell bodies. C and D, the signal intensity of CaMKIIα in neurons treated with DMSO or rapamycin was measured as a ratio over eGFP. Note that CaMKIIα protein decreases with rapamycin treatment. *, p < 0.05; ***, p < 0.005 by unpaired Student’s t test. Primary (1st) branch: DMSO, n = 21; Rapa, n = 24; secondary (2nd) branch: DMSO, n = 72; Rapa, n = 71. E, BVI was determined by normalizing all signals by eGFP within the same branch and using the following equation: BVI = [Daughter branch A – Daughter branch B]/Averaged control BVI. Note that CaMKIIα BVI is reduced when mTORC1 is inhibited with rapamycin. ***, p < 0.005 by unpaired Student’s t test. CaMKIIα: DMSO, n = 33 paired daughter branches; Rapa, n = 29 paired daughter branches. F, eGFP-expressing cultured hippocampal neurons were treated with vehicle or APS and immunostained for CaMKIIα. Representative neurons are shown. CaMKIIα images were also pseudocolored, and three-dimensional rendering was achieved using the Interactive 3D Surface Plot plug-in in ImageJ. Scale bar, 10 μm. G, the signal intensity of CaMKIIα in the cell body of neurons treated with vehicle or APS was measured as a ratio over eGFP. Vehicle, n = 16 neuronal cell bodies; APS, n = 14 neuronal cell bodies. H, the signal intensity of CaMKIIα in neurons treated with vehicle or APS was measured as a ratio over eGFP 10 μm before the branch point. Note that CaMKIIα protein decreases with APS treatment. *, p < 0.05 by unpaired Student’s t test. Vehicle, n = 14 primary branches; APS, n = 12 primary branches. J, the signal intensity of CaMKIIα in neurons treated with vehicle or APS was measured as a ratio over eGFP 10 μm after the branch point. ***, p < 0.01 by unpaired Student’s t test. Vehicle, n = 28 branches; APS, n = 26 branches. J, BVI was determined by normalizing all signals by eGFP within the same branch and using the following equation: BVI = [Daughter branch A – Daughter branch B]/Averaged control BVI. Note that CaMKIIα BVI is reduced when NMDAR is inhibited with APS. *, p < 0.05 by unpaired Student’s t test. Vehicle, n = 14 paired daughter branches; APS, n = 11 paired daughter branches. Error bars represent S.E.

the branch-specific targeting of CaMKIIα mRNA and 2) how does inhibition of mTORC1 reduce it? By first determining the mechanism that reduces branch-specific mRNA targeting, we might glean insight into the factors that mediate the process. In yeast, inhibition of TORC1 accelerates the deadenylation-decapping pathway (27). mRNAs that decay rapidly in the presence of rapamycin have shorter poly(A) tails possibly through rapid deadenylation (27). Thus, we hypothesized that deadenylation of CaMKIIα mRNA underlies the reduced CaMKIIα mRNA when mTORC1 is inhibited. Using the poly(A) tail length assay, we measured CaMKIIα mRNA poly(A) tail length when mTORC1 kinase was active or inhibited by rapamycin.
CaMKII

HuD is required for the branch-selective expression of CaMKII.

Dimensional rendering was achieved using the Interactive 3D Surface Plot plug-in in ImageJ to demonstrate change in signal intensity.

The same color to the right of CaMKII sites from the 3'-UTR of CaMKII. Note that the dendritic expression of CaMKII increases with mTORC1 inhibition.

Kv1.1 mRNA levels remained roughly the same with mTORC1 inhibition (Fig. 5C), whereas the poly(A) tail length remained relatively the same (5), whereas the poly(A) tail length remained relatively the same with mTORC1 inhibition (Fig. 5C). These results favor the hypothesis that the mTORC1-dependent reduction in CaMKIIβ mRNA is mediated by its rapid deadenylation and subsequent mRNA degradation.

The reduction in poly(A) tail length, band intensity (Fig. 5A), and sensitivity to RNase H treatment (Fig. 5B) all indicate that inhibiting mTORC1 activity shortens the CaMKIIβ mRNA poly(A) tail. Notably, Kv1.1 mRNA levels remained roughly the same (5), whereas the poly(A) tail length remained relatively the same with mTORC1 inhibition (Fig. 5C). These results favor the hypothesis that the mTORC1-dependent reduction in CaMKIIβ mRNA is mediated by its rapid deadenylation and subsequent mRNA degradation.

The Binding of the RNA-binding Protein HuD to the 3'-UTR of CaMKIIβ Is Required For Its Branch-selective Expression—Because the branch-specific expression of Kv1.1 was not affected by mTORC1 activity, we considered the possibility that HuD, an RNA-binding protein that binds to both mRNAs, could mediate branch-specific expression of CaMKIIβ. We recently demonstrated that HuD/CaMKIIβ mRNA interaction mediates the mTORC1-dependent expression of CaMKIIβ protein (5). Furthermore, we showed that CaMKIIα mRNA and Kv1.1 mRNA compete for HuD binding with CaMKIIβ mRNA “winning” when mTORC1 is active due to the higher affinity for HuD and abundance of CaMKIIα (5). In agreement with our data suggesting that reduced mTORC1 activity leads to the shortening of the CaMKIIβ mRNA poly(A) tail (Fig. 5), HuD stabilized its target mRNAs by delaying the onset of mRNA degradation and had an ~10-fold higher affinity for mRNAs with long poly(A) tails (>150 nucleotides) (18, 28, 29). Thus, if HuD is required for the branch-specific expression of CaMKIIα, then we would predict that deletion of HuD binding sites from the 3'-UTR of CaMKIIα would eliminate the polarized expression of CaMKIIα. Because CaMKIIα mRNA has 35 HuD binding sites (30), we turned to a reporter construct coding for myr-dGFP fused to the dendritic targeting sequence within the 3'-UTR of CaMKIIα that contains eight overlapping HuD binding sites (5, 23). As expected, neurons that expressed this reporter construct showed selective expression of myr-dGFP in one daughter branch over the other, having a BVI of ~1 (Fig. 6, A and B). In contrast, when we expressed this reporter construct with the HuD binding sites removed, the myr-dGFP signal seemed to accumulate at the branch point, reducing the BVI by 55% (Fig. 6, C and D). These results suggest that HuD mediates the branch-selective expression of CaMKIIα mRNA.

Knockdown of Endogenous HuD Reduces the Branch-selective Expression of CaMKIIα—As a further test to assess the relative importance of HuD in mediating the branch-specific expression of CaMKIIα in neurons, we transfected neurons with an shRNA designed and characterized to reduce or knock down (KD) HuD mRNA expression (22). To verify that the shRNA was effective at reducing HuD mRNA expression, we performed fluorescence in situ hybridization using an antisense probe set specific for HuD mRNA. As expected, only neurons transfected with eGFP and the HuD shRNA showed reduced HuD mRNA in the cell body (Fig. 7A, right panel, white arrow indicating nontransfected cells versus outlined transfected cell body). Furthermore, HuD mRNA was reduced by ~66% 72 h post-transfection in HuD KD neurons when compared with eGFP-expressing neurons that were transfected with vector

**FIGURE 3. Branch-specific expression of Kv1.1 does not require mTORC1 activity.** A, representative images of neurons treated with DMSO (control) or rapamycin and immunostained for Kv1.1. Colored arrows refer to the corresponding blown-up region of the dendrite that is outlined with the dotted line in the same color to the right. Note that the dendritic expression of Kv1.1 increases with mTORC1 inhibition. Kv1.1 images were also pseudocolored, and three-dimensional rendering was achieved using the Interactive 3D Surface Plot plug-in in ImageJ to demonstrate change in signal intensity. Scale bar, 10 μm. 10-D, quantification of Kv1.1 signal intensity normalized by eGFP in the cell body (B) as well as the primary (1°) (C) and secondary (2°) dendrites (D). The signal intensity of Kv1.1 in neurons treated with DMSO or rapamycin was measured as a ratio over eGFP 10 μm before or after the branch point. *, p < 0.05 by unpaired Student’s t test. Cell body: DMSO, n = 11; Rapa, n = 9; primary branch: DMSO, n = 11; Rapa, n = 9; secondary branch: DMSO, n = 24; Rapa, n = 18. E, BVI was determined by normalizing all signals by eGFP within the same branch and using the following equation: BVI = [Daughter branch A – Daughter branch B]/Averaged control BVI. Note that Kv1.1 expression in secondary dendrites is polarized in both control and rapamycin-treated neurons. Kv1.1: DMSO, n = 12 paired daughter branches; Rapa, n = 9 paired daughter branches. Error bars represent S.E.
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**FIGURE 4.** mTORC1 activity leads to branch-specific expression of CaMKIIα mRNA that is reduced with mTORC1 inhibition. **A,** FISH using the CaMKIIα mRNA-specific antisense probe. eGFP staining was used to outline the dendrites of the representative neurons shown. Colored arrows refer to the corresponding blown up region of the dendrite that is outlined with the dotted line in the same color to the right. **B,** FISH using a sense probe (negative control) to verify the specificity of the antisense probe used in **A.** For visualization purposes, the images showing the mRNA puncta were dilated once. **C,** CaMKIIα mRNA punctum number was measured in the cell body of DMSO- and rapamycin-treated neurons and normalized by area. DMSO, n = 25 neuronal cell bodies, 2000 puncta; Rapa, n = 21 neuronal cell bodies, 1544 puncta. **D,** CaMKIIα mRNA punctum number was measured in the primary (1°) branch of DMSO- and rapamycin-treated neurons. Note that punctum number is reduced with rapamycin treatment. **E,** CaMKIIα mRNA punctum number was measured in the secondary (2°) branch of DMSO- and rapamycin-treated neurons. Note that punctum number is reduced with rapamycin treatment. **F,** branch variability was determined by counting the number of CaMKIIα mRNA puncta in each daughter branch. Quantification of the difference in punctum number between the two branches is shown. **G,** CaMKIIα mRNA punctum signal intensity was measured in DMSO- and rapamycin-treated neurons from both the primary (10 μm before the branch point) and the secondary (10 μm after the branch point) branches. Error bars represent ± S.E.

alone (Fig. 7, **A** and **B**; control, 1.00 ± 0.21; HuD shRNA, 0.34 ± 0.12). As a negative control, we used a sense probe that did not detect any signal (Fig. 7C).

To determine whether HuD impacts the subcellular localization of CaMKIIα expression, we immunostained control and HuD shRNA-expressing neurons with an antibody against CaMKIIα. Notably, the cell body expression was highly variable with no significant overall change between groups of neurons. In contrast, the expression of CaMKIIα showed a downward trend in the primary dendrite and a significant decrease in the secondary branches in HuD shRNA-expressing neurons relative to control neurons transfected with vector alone (Fig. 7, **E–G**; primary dendrite: control, 1.00 ± 0.1; HuD shRNA, 0.68 ± 0.13; secondary dendrite: control, 1.00 ± 0.08; HuD shRNA, 0.87 ± 0.18). Similar to what we observed with our reporter construct, the branch-specific expression of CaMKIIα was dramatically reduced by ~56% in HuD KD neurons (Fig. 7G). Thus, a decrease in HuD expression results in reduced CaMKIIα polarized expression with more protein being distributed between daughter branches.

**HuD Targets One Daughter Branch over the Other in a Single Neuron**—We next examined the possibility that HuD itself may be selectively targeted to one daughter branch over the other and hence mediate the branch-specific expression of CaMKIIα when mTORC1 is active. Because the antibodies available to detect HuD are not reliable for immunofluorescence, we measured HuD protein with an anti-myc antibody directed against the overexpressed myc-tagged HuD protein in hippocampal neurons. Unlike CaMKIIα, total myc-HuD levels did not change with mTORC1 activity as indicated by the quantification of signal in both the primary and the secondary branches (Fig. 8, **A–C**). Surprisingly, the BVI of myc-HuD more than doubled upon mTORC1 inhibition (HuD, DMSO, 1.00 ± 0.16; HuD, Rapa, 2.43 ± 0.37; Fig. 8D).
To ensure that HuD overexpression does not increase the diameter of one daughter branch over the other, thus favoring increased protein expression in the larger branch over the smaller branch by diffusion, we measured the diameter of both daughter branches using eGFP in control and HuD-overexpressing neurons. There was no significant difference in the diameter between daughter branches within the same neuron when comparing control and HuD-expressing neurons (Fig. 8E). Collectively, these results suggest that HuD may direct the branch-specific expression of its target mRNAs.

HuD Rescues CaMKIIα Protein Expression and Branch Variability When mTORC1 Is Inhibited—Next, we examined the possibility that overexpression of HuD would increase CaMKIIα protein and hence restore its BVI when mTORC1 is inhibited. We predicted that CaMKIIα BVI would be maintained in HuD-overexpressing neurons in the presence of rapamycin due to the increased targeting of HuD to one daughter branch over the other. Similar to what was observed in Fig. 2, CaMKIIα protein levels in the cell body in HuD-overexpressing neurons relative to control neurons remained the same regard-
less of mTORC1 activity (Fig. 8F). As predicted, in contrast to the cell body, HuD-overexpressing neurons had significantly more CaMKIIα protein in the primary dendrite when mTORC1 was inhibited by rapamycin (Fig. 8, A and G). Furthermore, in rapamycin-treated neurons, HuD restored CaMKIIα expression back to control levels in the secondary branches (Fig. 8, A and H). Consistent with the increased targeting of HuD when mTORC1 activity was reduced, HuD restored CaMKIIα BVI back to control levels (Fig. 8, A and I). These results suggest that HuD increases CaMKIIα protein and maintains its BVI when mTORC1 is inhibited.

Branch-specific Expression of CaMKIIα Requires the Poly(A) Tail-binding RNA Recognition Motif of HuD—If HuD protects CaMKIIα mRNA from deadenylation in a branch-specific manner, then expressing a truncated form of HuD that does not bind to the poly(A) tail of its targets (18, 28, 29) will not rescue CaMKIIα BVI. HuD has three RNA recognition motifs (RRMs), two of which bind specific HuD binding motifs in the mRNA sequence of its targets. The third RRM binds the poly(A) tail of its mRNA targets (28, 29). Because shortening of the CaMKIIα poly(A) tail led to mRNA degradation, we examined whether the third RRM and linker region of HuD are required to mediate the HuD-dependent rescue of CaMKIIα BVI when mTORC1 is inhibited. Indeed, overexpression of a HuD construct lacking the third RRM and linker region of HuD is required to mediate poly(A) tail binding and BVI induction (Figs. 8, A and I). Interestingly, in HuD1+II-expressing neurons, CaMKIIα levels were high when mTORC1 was active, and the expression of HuD remained polarized (myc-HuD, Fig. 8, C and D; CaMKIIα, Fig. 8, G and H). Unexpectedly, the ability of HuD to mediate branch-specific expression of CaMKIIα was greatly reduced (Fig. 8, A and I, red

FIGURE 7. Knockdown of endogenous HuD decreases the branch-specific expression of CaMKIIα. A, representative images of neurons co-transfected with control vector or shRNA against HuD and eGFP cDNA. 72 h post-transfection, neurons were fixed, and HuD mRNA was detected using an antisense probe set against HuD mRNA. Notice the strong detection of HuD mRNA-containing granule structures in the cell bodies of the control eGFP neuron (left, white dotted outline of cell body) compared with the HuD shRNA (right, white dotted outline of cell body) eGFP neuron. Note that untransfected neurons in the HuD KD panel still express HuD mRNA as indicated by red in situ signal (white arrows). For visualization purposes, the images showing the mRNA puncta were dilated twice. B, quantification of the -fold change of reduced HuD mRNA-containing puncta per area as determined by eGFP signal in the cell body of control (Cont) and shRNA-expressing neurons. Note the ~63% reduction in HuD mRNA detection in shRNA-expressing neurons. C, representative neurons showing specificity of the HuD antisense probe set. Note the red in situ punctum signal only with the antisense probe set. D, representative images of CaMKIIα expression in control and HuD shRNA-expressing neurons. Colored arrows refer to the corresponding blown up region of the dendrite that is outlined with the dotted line in the same color to the right. CaMKIIα images were also pseudocolored, and three-dimensional rendering was achieved using the Interactive 3D Surface Plot plug-in in ImageJ. E, the signal intensity of CaMKIIα in the cell body of control and HuD shRNA-expressing neurons measured as a ratio over eGFP. F, quantification of CaMKIIα expression in the primary (1) branch 10 μm prior to the branch point. G, quantification of CaMKIIα expression in the secondary (2) branch. H, BVI for control and HuD shRNA-expressing neurons. Note the significant reduction in BVI in HuD shRNA neurons compared with control neurons. Significance was determined by Student’s t test. *, p < 0.05. Error bars represent S.E.
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FIGURE 8. Differential branch expression of full-length myc-HuD but not its truncated form missing the domain that binds the poly(A) tail rescues CaMKIIα branch variability when mTORC1 is inhibited. A, top, schematic showing HuD constructs used. Cultured hippocampal neurons were transfected with pcDNA+eGFP, pchHuD+eGFP, or pchHuDI+II+eGFP and treated with either carrier (DMSO) or rapamycin. Representative neurons immunostained for CaMKIIα and myc-HuD are shown. Dotted box indicates blown up branch points to the right. Scale bar represents 10 μm. CaMKIIα and myc-HuD images were also pseudocolored, and three-dimensional rendering was achieved using the Interactive 3D Surface Plot plug-in in ImageJ. A yellow arrows indicates a daughter branch with more HuD and CaMKIIα protein relative to the other daughter branch (white arrow). Scale bar, 10 μm. B, primary (10 μm before branch point) dendritic myc-HuD protein was measured as a ratio over eGFP. HuD DMSO, n = 11 primary dendrites; HuD Rapa, 13 primary dendrites; HuD I+II DMSO, n = 15 primary branches; HuD I+II Rapa, n = 10 primary branches. **, p < 0.01; ***, p < 0.005 by one-way ANOVA Newman-Keuls post-test. C, myc-HuD protein in secondary daughter branches (10 μm after the branch point) was measured as a ratio over eGFP. HuD DMSO, n = 22 secondary branches; HuD Rapa, n = 26 secondary branches; HuD I+II DMSO, n = 30 secondary branches; HuD I+II Rapa, n = 20 secondary branches. ***, p < 0.005 by one-way ANOVA Newman-Keuls post-test. D, myc-HuD/eGFP protein was subtracted between daughter branches and normalized to myc-HuD DMSO and graphed as BVI. Note the increased BVI for myc-HuD between daughter branches in rapamycin-treated neurons. **, p < 0.01 by one-way ANOVA Newman-Keuls post-test. DMSO, n = 15 paired daughter branches. HuD I+II Rapa, n = 26 paired secondary branches; HuD I+II DMSO, n = 15 paired daughter branches; HuD I+II Rapa, n = 10 paired daughter branches. E, the difference in diameter between daughter branches of neurons transfected with eGFP+pcDNA or eGFP+myc-HuD was measured using eGFP. Note there is no significant difference in branch diameter between pcDNA and myc-HuD neurons, pcDNA, n = 10 secondary branches; HuD, n = 20 secondary branches. F, CaMKIIα protein was measured in the cell body of HuD- or HuD I+II-transfected neurons that were then DMSO- or rapamycin-treated. HuD DMSO, n = 27; HuD Rapa, n = 24; HuD I+II DMSO, n = 16; HuD I+II Rapa, n = 15. *, p < 0.05 by one-way ANOVA Newman-Keuls post-test. G, primary dendritic CaMKIIα protein (10 μm before branch point) was measured as a ratio over eGFP. Note that HuD rescues reduced CaMKIIα levels in rapamycin-treated neurons. #, p < 0.05; ##, p < 0.01 significantly different from pcDNA DMSO by one-sample t-test. *, p < 0.05; ***, p < 0.005 by one-way ANOVA Newman-Keuls post-test. H, primary dendritic HuDI+II protein (10 μm before branch point) was measured as a ratio over eGFP. HuD DMSO, n = 36 paired secondary branches; HuD Rapa, n = 34 paired secondary branches; HuD I+II DMSO, n = 36 secondary branches; HuD I+II Rapa, n = 30 secondary branches. I, the absolute value of the difference between CaMKIIα/eGFP protein was subtracted between daughter branches and normalized to pcDNA DMSO BVI and graphed as BVI. Note that HuD rescues reduced CaMKIIα BVI when mTOR is inhibited. In addition, removing the linker region and third RRM significantly reduces the branch-specific expression of CaMKIIα when mTORC1 is active (red arrow). ##, p < 0.01, significantly different from DMSO control, as determined by a single t-test. *, p < 0.05; **, p < 0.01 by one-way ANOVA Newman-Keuls post-test. HuD DMSO, n = 36 paired daughter branches; HuD Rapa, n = 44 paired daughter branches; HuD I+II DMSO, n = 18 paired daughter branches; HuD I+II Rapa, n = 15 paired daughter branches. Error bars represent S.E.
**FIGURE 9.** Fluorescence recovery after photobleaching of myristoylated dGFP-CaMKIIα-UTR demonstrates that HuD binding sites facilitate branch-specific local translation. A. Representative images of dendrites expressing myr-dGFP fused to CaMKIIα-UTR in which HuD binding sites are maintained (HuD; left) or deleted (ΔHuD; right). dGFP fluorescence of the same dendrites before (top), immediately after (0 min), and 30 min after photobleaching. The bottom panel is an overlay of dGFP at 0 (green) and 30 min (red). Yellow indicates unbleached dGFP. After 30 min of recovery, myr-dGFP-CaMKIIα-UTR HuD displays polarized translation of dGFP, whereas myr-dGFP-CaMKIIα-UTR ΔHuD shows approximately equal dGFP translation. Red arrowheads indicate GFP signals that disappear or are reduced immediately after bleaching (time 0) and reappear 30 min after recovery; dGFP signals are overlaid on their respective dendrites (gray). Scale bar, 10 μm. B and C, deletion of HuD binding sites does not affect the total expression of new myr-dGFP in primary (HuD) (0.00 ± 0.05; ΔHuD: 0.14 ± 0.05; p = 0.35) and secondary (C) (HuD: 0.10 ± 0.03; ΔHuD: 0.35 ± 0.05; p = 0.05) dendrites 30 min after bleaching. D, deletion of HuD binding sites reduces polarized translation between daughter dendrites that emerge from a single primary dendrite. Dendrite A is assigned to a daughter dendrite that expresses more dGFP, and dendrite B is assigned to a daughter dendrite that expresses less dGFP. Data points of designated A and B daughter dendrites in a single neuron are connected by dotted lines (HuD: A = 1.00 ± 0.17; HuD B = 0.26 ± 0.08; ΔHuD A = 0.44 ± 0.09; ΔHuD B = 0.30 ± 0.08). At 30 min after recovery, HuD daughter dendrites display more polarized expression of new myr-dGFP than ΔHuD daughter dendrites (HuD A = 0.00 ± 0.00; ΔHuD A = 0.14 ± 0.05; p = 0.006; one-way ANOVA, Tukey’s multiple comparison test). However, dendrite A, a daughter dendrite conventionally assigned as expressing more dGFP, and dendrite B, a daughter dendrite designated as expressing less myr-dGFP, exhibited similar levels of new myr-dGFP protein, regardless of the presence of HuD binding sites (HuD A = 0.26 ± 0.08; ΔHuD B = 0.30 ± 0.08; Fig. 9C). Additionally, the levels of new myr-dGFP between HuD daughter dendrites A and B are significantly polarized compared with ΔHuD daughter dendrites (HuD A-B, p < 0.0001; ΔHuD A-B, p = 0.8389; one-way ANOVA, Tukey’s multiple comparison test). These findings suggest that between daughter branches the presence of HuD binding sites generally supports the polarized, dendritic translation of myr-dGFP-CaMKIIα mRNA.

For both reporters, we detected comparable syntheses of new myr-dGFP proteins at 6 min after photobleaching as we saw a significant increase in fluorescence compared with baseline (F₀). The difference in new myr-dGFP synthesis between +HuD daughter dendrites remarkably increased throughout the recovery period, demonstrating that one daughter dendrite has a higher compared with baseline (dashed line). Baseline fluorescence intensity immediately after photobleaching (time = 0 min; F₀). Removing HuD binding sites abrogates the polarized new translation of myr-dGFP between daughter dendrites (HuD slope: 0.40 ± 0.01; ΔHuD slope: 0.01 ± 0.06). Slopes were determined by linear regression analysis (r, p < 0.003). 30 min after photobleaching, HuD daughter dendrites exhibit greater polarity in myr-dGFP expression compared with ΔHuD daughter dendrites (HuD = 114.5 ± 2.4%; ΔHuD = 106.1 ± 1.0%; *p < 0.003). F, deletion of HuD binding sites reduces dendritic BVI of new myr-dGFP protein after 30 min of recovery (HuD = 1.00 ± 1.5; ΔHuD = 0.48 ± 0.1; p < 0.007). n = 11 neurons for myr-dGFP-CaMKIIα-UTR HuD. n = 10 for myr-dGFP-CaMKIIα-UTR ΔHuD. Student’s t-test was used for statistical analyses for B, C, and E. Error bars represent S.E. 1°, primary; 2°, secondary. *, p < 0.05; **, p < 0.01; ****, p < 0.001. 

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These findings suggest that binding of the poly(A) tail to the third RRM of HuD underlies the differential expression of CaMKIIα between daughter branches.

Branch-specific Local Translation of a CaMKIIα Reporter Requires HuD mRNA Binding Sites—Because HuD mediated the polarized expression of CaMKIIα mRNA, we hypothesized that CaMKIIα mRNA local translation is also branch-specific. To test our hypothesis, we performed fluorescence recovery after photobleaching of our reporters, myristoylated dGFP fused to the 3’-UTR of CaMKIIα in which HuD binding sites were maintained (myr-dGFP-CaMKIIα UTR HuD) or removed (myr-dGFP-CaMKIIα UTR ΔHuD) (Fig. 6) (23, 24, 31). We then measured the translation-dependent recovery from photobleaching of each reporter construct. An increase in fluorescence during recovery represents newly synthesized dGFP protein (Fig. 9A, red arrows) (31). Because dGFP contains a myristoylation site, which tethers the protein to the membrane and thus limits protein movement, increased dGFP fluorescence is due to local protein synthesis and not protein diffusion (23, 24, 31). Removal of HuD binding sites did not alter the total expression of new myr-dGFP in the primary and secondary dendrites at 30 min of recovery (primary: HuD = 1.00 ± 0.40, ΔHuD = 1.48 ± 0.31; secondary: +HuD = 1.00 ± 0.23, ΔHuD = 0.83 ± 0.12; Fig. 9g, B and C). However, dendrite A, a daughter dendrite conventionally assigned as expressing more dGFP, with the HuD binding sites contained more dGFP than dendrite A without the HuD binding sites (HuD A = 1.00 ± 0.17; ΔHuD A = 0.44 ± 0.09; Fig. 9D). Dendrite B, a daughter dendrite designated as expressing less myr-dGFP, exhibited similar levels of new myr-dGFP protein, regardless of the presence of HuD binding sites (+HuD B = 0.26 ± 0.08; ΔHuD B = 0.30 ± 0.08; Fig. 9C). Additionally, the levels of new myr-dGFP between HuD daughter dendrites A and B are significantly polarized compared with ΔHuD daughter dendrites (HuD A-B, p < 0.0001; ΔHuD A-B, p = 0.8389; one-way ANOVA, Tukey’s multiple comparison test). These findings suggest that between daughter branches the presence of HuD binding sites generally supports the polarized, dendritic translation of myr-dGFP-CaMKIIα mRNA.
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One of the most surprising results herein is that the absence of HuD binding to the poly(A) tail resulted in the equal distribution of CaMKIIα protein in both daughter branches, suggesting that poly(A) binding is required to mediate CaMKIIα branch-specific expression. These results lead to the intriguing possibility that the length of the poly(A) tail of plasticity-related mRNAs may serve as the bait for HuD capture and branch-selective expression.

Redondo and Morris (15) have suggested that there may be multiple tags that can facilitate synaptic capture of plasticity-related proteins. Consistent with this idea, previous reports have suggested that CaMKIIα itself serves as a tag. Notably, inhibiting the phosphorylation of CaMKIIα prevents late phase LTP, a requirement to serve as a tag (40). Although we did not detect CaMKIIα in both branches, blocking CaMKIIα activity in activated synapses where it is localized may be sufficient to block long term changes in plasticity. Through the discovery of HuD as the RNA-binding protein that mediates CaMKIIα expression herein, future experiments may help elucidate the tag/plasticity-related protein interactions in more complex systems.

The question of how specific mRNAs target activated synapses is perplexing. It has been suggested that neuronal ribonucleoproteins patrol a group of synapses (41). Consistent with this idea, bidirectional movement of mRNAs in dendrites has been observed (41–45). Global mRNA “exploration” may be required for the local protein synthesis at stimulated synapses during early events that set the stage for long term plasticity (46, 47). Interestingly, HuD protein levels increase with neuronal/mTORC1 activity (5, 48), and the protein is targeted in a branch-specific manner. Collectively, these data suggest that HuD is a good candidate to target the mRNAs coding for proteins required to strengthen neighboring synapses to facilitate late stage plasticity.

In summary, our previous study demonstrating that HuD can switch target mRNAs from CaMKIIα when mTORC1 is active to Kv1.1 when mTORC1 is inhibited combined with these findings suggests that the branch-specific expression of HuD may be what “captures” mRNAs to specifically shuttle and stabilize them in one daughter branch based on their affinity and abundance. How HuD protein targets one daughter branch over the other in a single neuron is yet to be determined. However, what is clear is that the mRNA that it captures, be it CaMKIIα mRNA when mTORC1 is active or Kv1.1 mRNA when mTORC1 activity is reduced, will depend on the level of mTORC1 activity, serving as the tag and dictating the strength of the synapse (5, 41).

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