A Role for a Rat Homolog of Staufen in the Transport of RNA to Neuronal Dendrites

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Summary

RNAs are present in dendrites and may be used for local protein synthesis in response to synaptic activity. To begin to understand dendritic RNA targeting, we cloned a rat homolog of *staufen*, a *Drosophila* gene that participates in mRNA targeting during development. In hippocampal neurons, rat staufen protein displays a microtubule-dependent somatodendritic distribution pattern that overlaps with dendritic RNAs. To determine whether r-staufen is required for dendritic RNA targeting, we constructed a mutant version containing the RNA binding domains (stau-RBD) but lacking the C-terminal portion potentially involved in dendritic targeting. Stau-RBD expression was restricted to the cell bodies and proximal dendrites. Expression of stau-RBD significantly decreased, while overexpression of wild-type r-staufen increased, the amount of dendritic mRNA. Taken together, these results suggest that the rat staufen protein plays an important role in the delivery of RNA to dendrites.

Introduction

Anatomical observations of translation machinery (Steward and Levy, 1982) and mRNAs in neuronal dendrites have raised the possibility that synapses make use of locally synthesized proteins (Schuman, 1999). Indeed, recent studies have demonstrated that dendritic protein synthesis can accompany plasticity (Ouyang et al., 1999; Sheetz et al., 2000; Wu et al., 1998) and may participate in long-lasting changes in synaptic strength (Casadio et al., 1999; Huber et al., 2000; Kang and Schuman, 1996; Martin et al., 1997; Raymond et al., 2000). In hippocampal slices, three different forms of synaptic plasticity appear to require local protein synthesis. The potentiation induced by BDNF is prevented by the coapplication of a protein translation inhibitor (Kang and Schuman, 1996). Furthermore, in slices where the synaptic neuropil is isolated from both pre- and postsynaptic cell bodies, the protein synthesis dependence persists (Kang and Schuman, 1996), indicating that the source of new proteins cannot be the pre- or postsynaptic cell bodies. Experiments similar to those described above indicate that long-term depression (LTD) induced by metabotropic receptor (mGluR) activation in hippocampal slices also requires protein synthesis (Huber et al., 2000). The activation of mGluRs before a tetanus can also facilitate long-term potentiation (LTP) induction and persistence, a phenomenon known as "priming" (Cohen and Abra-

ham, 1996; Cohen et al., 1998). In this case, the application of a protein synthesis inhibitor during mGluR activation prevents the priming effect (Raymond et al., 2000). Given that the mGluR-priming effect occurs quickly (<20 min), these data implicate the dendrites as the source of newly synthesized proteins. This idea is supported by the demonstration that mGluR agonists can stimulate protein synthesis in synaptoneurosome preparations (Weiler and Greenough, 1997).

If dendritic protein synthesis influences synaptic function and plasticity, then it is important to understand the regulation of mRNA delivery to the dendrites. Simple evidence for the regulated delivery of mRNAs is provided by clear differences in the distribution pattern of distinct mRNAs found in the same neurons. For example, MAP2 mRNA appears in the proximal one-third of dendrites (Garner et al., 1988) whereas αCAMKII mRNA is found throughout the dendritic arbor (Burgin et al., 1990). In addition, synaptic plasticity can alter the trafficking of mRNAs to the dendrites (Knowles and Kosik, 1997; Righi et al., 2000; Steward et al., 1998; Tongiorgi et al., 1997). In the dentate gyrus, Arc mRNA induced by electroconvulsive shock can be selectively targeted or stabilized at activated synapses (Steward et al., 1998). In cultured hippocampal neurons, treatment with NT-3 resulted in the movement of RNA-containing granules into more distal aspects of the dendrites (Knowles and Kosik, 1997). In addition, depolarization of neurons (Tongiorgi et al., 1997) or treatment with BDNF (Righi et al., 2000) increased the amount and extent of both BDNF and TrkB mRNA found in the dendrites.

Despite clear evidence that mRNA trafficking can be regulated by synaptic activity, we know relatively little about the mechanisms by which mRNAs are transported to dendrites. Many polarized cells, including neurons, contain asymmetrically distributed mRNAs. The mechanisms responsible for mRNA targeting may be at least partially conserved. For example, in both *Drosophila* oocytes (Pokrywka and Stephenson, 1991; Yisraeli et al., 1990) and mammalian neurons (Bassell et al., 1994), the transport of mRNAs is mediated by microtubules. This type of observation suggests the possibility that similar RNA binding proteins may participate in RNA delivery in neurons and other polarized cells. We have examined whether staufen, a protein known to be critical for the targeting of specific mRNAs in *Drosophila* oocytes and neuroblasts (Ephrussi et al., 1992; Ferrandon et al., 1994; Li et al., 1997; St. Johnston et al., 1991), participates in mRNA localization in neurons.

Results

### A Rat Homolog of Staufen Is Present in the Somata and Dendrites of Hippocampal Neurons
Using a mouse expressed sequence tag (EST) with high homology to *Drosophila* staufen as a probe, we cloned a rat homolog of *staufen* (*r-staufen*) from a rat hippocampal cDNA library. *R-staufen* encodes a protein with three predicted full-length double-stranded RNA binding do-
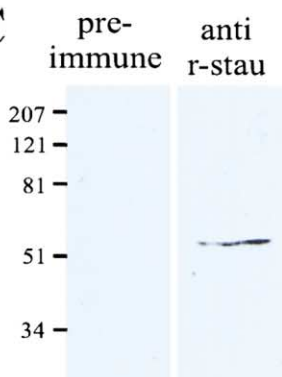
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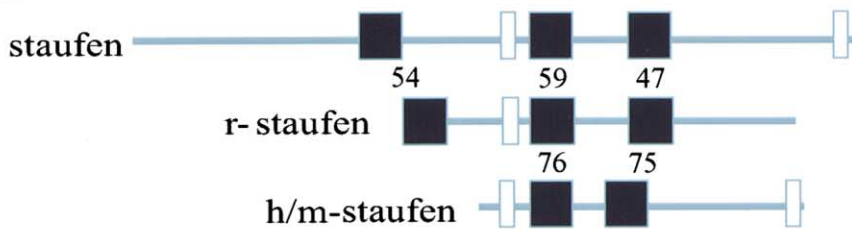
Figure 1. Cloning and Domain Structure of R-Staufen

(A) Amino acid sequence of rat staufen (r-stau 1) and alignment with *Drosophila* staufen (stau), two human staufen isoforms (h-stau 1 and 2), and a mouse staufen (m-stau 1). Conserved amino acids are boxed in black; similar amino acids are outlined. (B) Shown is a domain structure for r-staufen. The large black and small white boxes correspond to long and short double-stranded RNA binding domains (RBD), respectively (St. Johnston et al., 1992). Shown for comparison are the domain structures for *Drosophila* staufen (staufen) (St. Johnston et al., 1991) and a
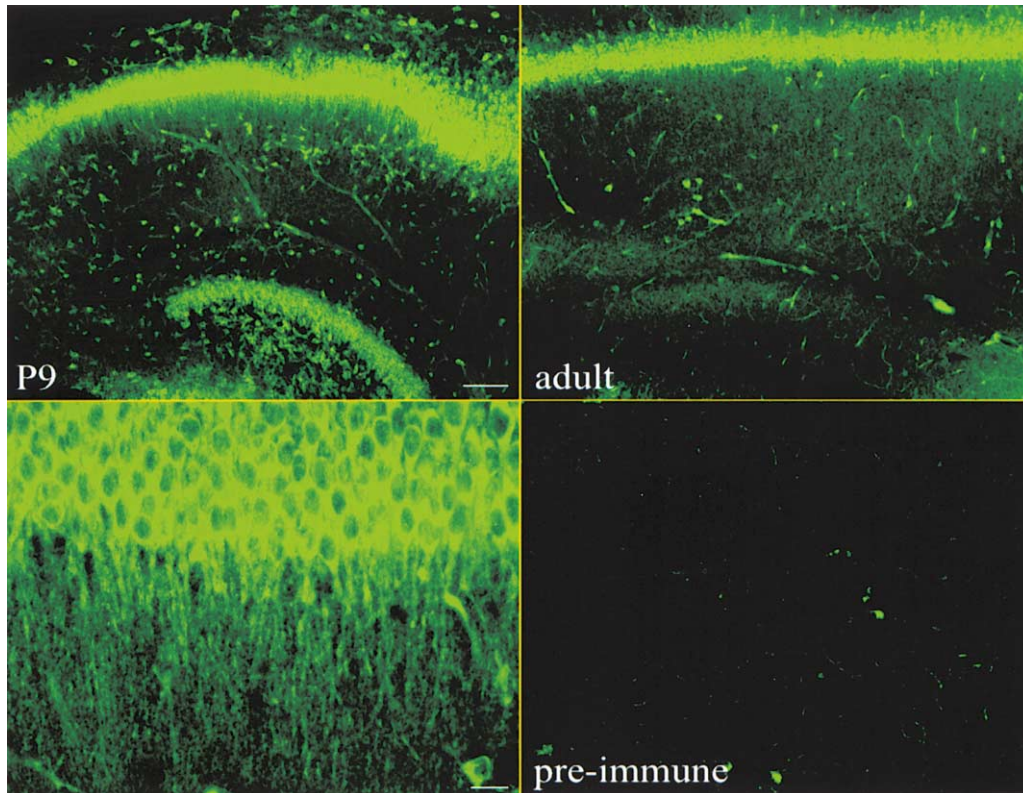
Figure 2. R-Staufen Is Present in the Soma and Dendrites of CA1 Pyramidal Neurons

Fluorescence immunohistochemistry showing staining for r-staufen in young (P9-) and adult (P54) hippocampal slices. R-staufen is abundant in both the soma (cytosol) and dendrites of CA1 pyramidal neurons. All images are 10×, except for the bottom left image which is 60× (P9). Background staining in the preimmune panel is due to blood vessels. Scale bars:100 and 20 μm, in the top and bottom images, respectively.

mains (RBD) (St Johnston, 1995; St. Johnston et al., 1992) showing 47%–59% similarity to the corresponding RBDs of *Drosophila* staufen (Figure 1); r-staufen is molecularly distinct from other recently identified mammalian staufen homologs, including a human or mouse homolog whose amino acid sequence and domain structure is shown in Figures 1A and 1B (Kiebler et al., 1999; Marion et al., 1999; Wickham et al., 1999). We raised an antibody to r-staufen; Western blot analysis revealed that the r-staufen protein is present in hippocampal homogenates and is detected as a single band by this antibody (Figure 1C). Fluorescence immunohistochemistry with the same r-staufen antibody revealed a punctate, somatodendritic distribution pattern for the protein in hippocampal slices and cultured hippocampal neurons (Figures 2 and 4C). In slices, r-staufen was observed throughout the dendrites of CA1 neurons in both stratum radiatum and stratum lacunosum moleculare (Figure 2). In addition, the cell bodies of putative interneurons as well as the granular and hilar cells of the dentate gyrus also appeared r-staufen positive. In cultured neurons, r-staufen-positive particles were evident throughout the dendritic tree (Figure 4C), similar

to what has been observed for a different mammalian staufen homolog (Kiebler et al., 1999).

Rat Staufen Is Delivered to Dendrites via a Microtubule-Based Network
Previous studies have suggested a role for microtubules, but not actin filaments, in the dendritic localization of mRNA (Bassell et al., 1994). We examined the localization of r-staufen protein following treatment with inhibitors of either the actin- or microtubule-based cytoskeleton (cytochalasin B or nocodozole, respectively). For this, we constructed an r-staufen-GFP (green fluorescent protein) fusion and used Sindbis virus to express the stau-GFP in hippocampal neurons. (In this and all subsequent experiments, neurons were analyzed approximately 12 hr post infection.) The localization of r-staufen-GFP was very similar to that observed for the endogenous r-staufen protein (Figure 3); the fusion protein was present in the soma and dendrites exhibiting a particularly punctate distribution in the distal dendrites. This dendritic localization of r-staufen was disrupted by nocodozole, but not cytochalasin B; nocodazole treatment did not result in the retraction of dendrites (Figure

recently cloned mouse or human staufen (h/m-staufen) (Marion et al., 1999; Wickham et al., 1999). Numbers indicate percent similarity between full-length RBDs of different staufen proteins. (C) Western blot of hippocampal homogenates probed with a polyclonal antibody raised against r-staufen. A single band is observed at ∼55 kDa.
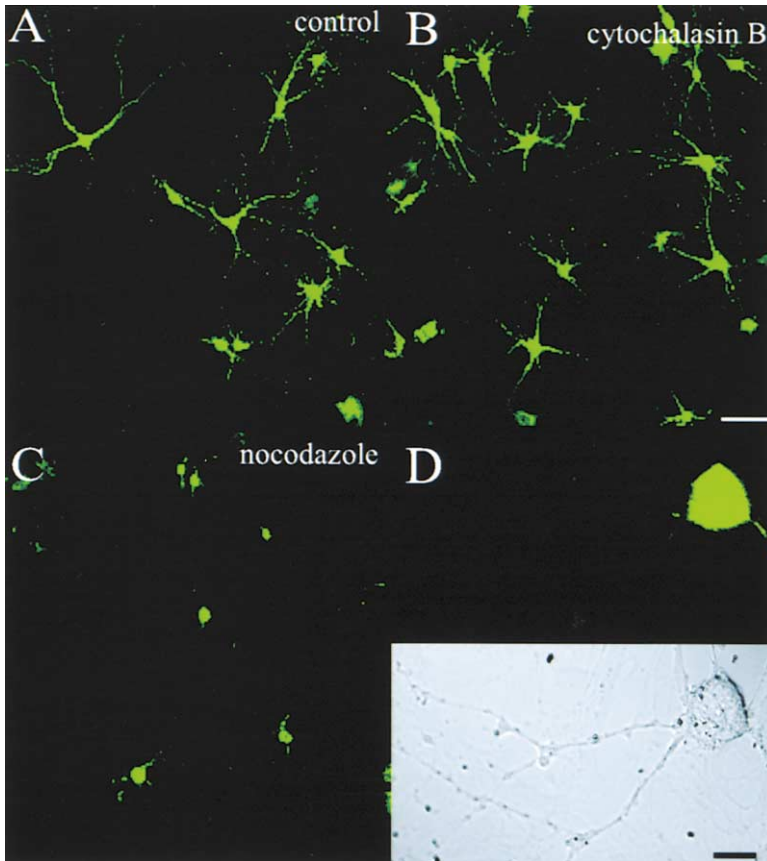
Figure 3. R-Staufen Is Delivered to Dendrites via a Microtubule-Based Network

Shown are low power confocal images of r-stau-GFP-expressing cultured hippocampal neurons. (A) In control media, staufen was distributed in the cell bodies and dendrites. (B) Treatment with cytochalasin B (2 μM) had no effect on the somatodendritic localization of r-staufen. (C) Treatment with nocodozole (20 μM) abolished the dendritic localization in all cells examined. (D) DIC and fluorescence image of a nocodazole-treated cell showing that the nocodazole blocked the dendritic localization of staufen but did not result in dendritic retraction. Scale bar: 20 μm.

3). These data indicate that r-staufen is actively transported via a microtubule-based system.

R-Staufen Protein Distribution Is Spatially Coincident with Dendritic RNA

The somatodendritic localization as well as the microtubule-based transport of r-staufen suggest a role in the trafficking of mRNAs. We next determined whether the distribution of r-staufen overlaps with RNA in hippocampal dendrites. We used ethidium bromide (EtBr) to fluorescently label total cellular RNA in cultured hippocampal neurons. Under the appropriate labeling conditions, EtBr revealed abundant RNA signal in the cell body and the dendrites, as indicated by overlap with simultaneous immunofluorescent labeling for the dendritic marker MAP2 (Figure 4A). The sensitivity of the dendritic EtBr signal to RNase, but not DNase treatment, indicated that under the appropriate experimental conditions, EtBr can be a specific reporter of RNA localization within neuronal dendrites (Figure 4A). In addition, EtBr labeling in dendrites overlaps with that SYTO 14, a stain that exhibits fluorescence enhancement upon binding nucleic acids (Knowles et al., 1996) (Figure 4B). We then examined the dendritic RNA distribution relative to r-staufen, using EtBr labeling combined with fluorescence immunohistochemistry. We found a strong colocalization of the RNA and r-staufen protein signals; this spatial coincidence was particularly evident in the distal aspects of the dendrites where there was an overlapping punctate distribution of both r-staufen and RNA (Figure

4C). These results are consistent with the hypothesis that rat staufen is associated with dendritic RNAs in vivo and may be involved in their targeting to dendritic compartments.

Expression of a Truncated Staufen Reduces the Dendritic RNA Pool

To examine directly whether r-staufen participates in the delivery of RNA to dendrites, we made a truncated r-staufen-GFP fusion protein (stau-RBD) which possesses all of the RNA binding domains of r-staufen but lacks the C-terminal portion of the protein adjacent to the last RBD which may be directly or indirectly involved in microtubule binding, as shown for other staufen proteins (Li et al., 1997; Wickham et al., 1999) (see Figure 1). As such, stau-RBD could act as a dominant negative, preventing the association of full-length staufen with RNA. We tested the ability of stau-RBD to inhibit full-length staufen mRNA association. As also shown by others (Wickham et al., 1999; Marion et al., 1999), we found that a full-length staufen-GST fusion protein could bind bicoid mRNA. The addition of stau-RBD reduced the mRNA association with staufen in a concentration-dependent manner [mean percent inhibition of full-length staufen-bicoid mRNA binding: 2:1 ratio of full-length staufen: stau-RBD = 25.9%; 1:1 ratio = 75.4%; 1:2 ratio = 89.5%]. We also hypothesized that stau-RBD, although capable of binding RNA, lacked the ability to transport RNA to the dendrites, owing to the C-terminal tail deletion. Indeed, this was the case: unlike full-length
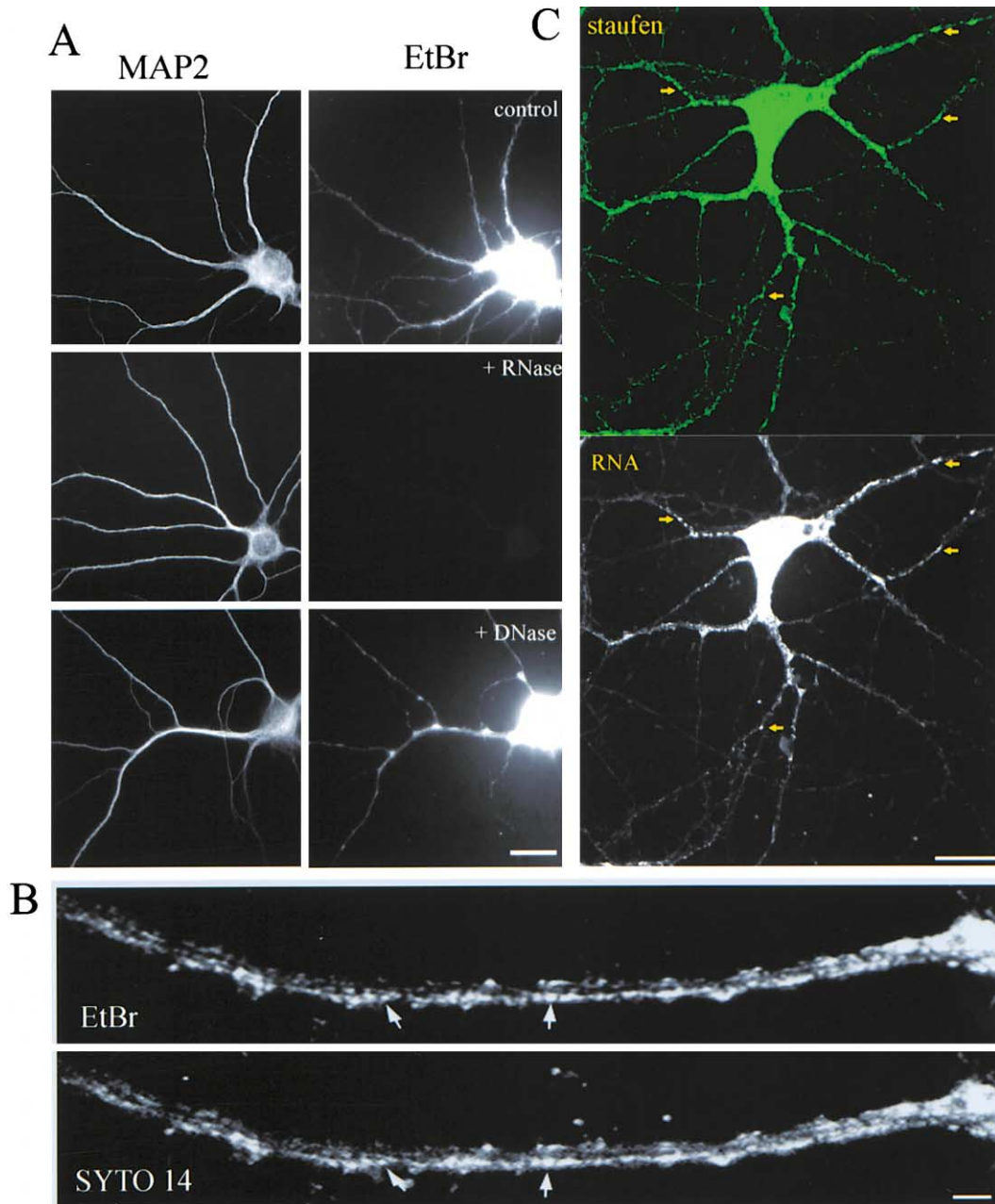
Figure 4. R-Staufen Colocalizes with RNA in Dendrites

(A) Images show MAP2 immunostaining (left) and ethidium bromide (EtBr) labeling (right) of individual, cultured hippocampal neurons (see Experimental Procedures). Treatment with EtBr effectively labels the cellular RNA pool as the EtBr signal is abolished by treatment with RNase, but not DNase. Scale bar: 20 μm. (B) EtBr signals in dendrites overlap with the nucleic acid stain SYTO 14. Shown is a principal dendrite stained with both SYTO 14 and EtBr. The overlap between the two signals suggests that both methods label the dendritic RNA pool. Scale bar: 5 μm. (C) R-staufen immunolabeling in cultured hippocampal neurons is colocalized with RNA in the dendrites. Arrows highlight some of the areas where the r-staufen and RNA signals clearly overlap. Scale bar: 10 μm.

r-staufen, the distribution of stau-RBD in cultured hippocampal neurons was limited to the soma and very proximal dendrites, as indicated by simultaneous immunostaining for MAP2 (Figure 5A). Note that the amount of stau-RBD protein present in infected neurons was statistically indistinguishable from that of the other GFP fusion proteins (e.g., stau-GFP and KH-GFP), indicating that its limited distribution is not due to lower protein

levels. In addition, we estimate that the concentration of stau-RBD in infected neurons is at least $10\times$–$20\times$ greater than the endogenous staufen in control GFP-expressing neurons (see Experimental Procedures).

Because stau-RBD was not efficiently transported to dendrites, we then asked whether its expression could reduce the amount of dendritic RNA, by potentially competing with endogenous r-staufen. We compared the
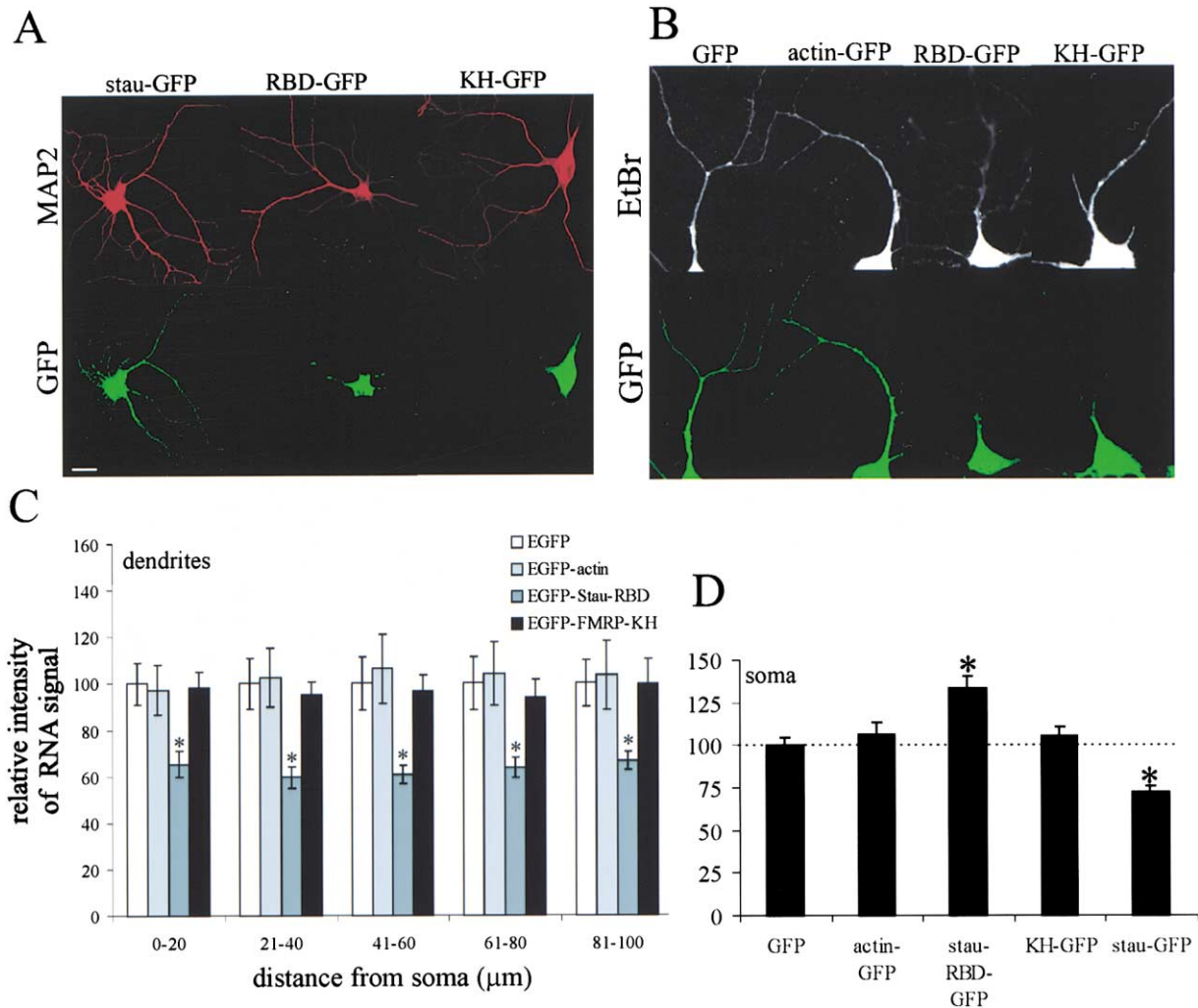
Figure 5. Expression of a Truncated R-Staufen Protein Reduces the Amount of RNA in Dendrites

(A) Green fluorescent protein fusions constructed with either full-length r-staufen (stau-GFP) or a truncated r-staufen (stau-RBD-GFP) result in distribution in the soma and dendrites of cultured hippocampal neurons, as indicated by immunolabeling for the dendritic protein MAP2. Stau-GFP was detectable throughout the dendritic arbor of neurons. In contrast, expression of stau-RBD-GFP was restricted to the soma, with dramatically lower but detectable signal in the proximal dendrite. A similar, somatically restricted pattern was observed for FMRP-KH-GFP. Scale bar: 15 μm. (B) Shown are both the GFP and EtBr signal for four different representative neurons expressing either GFP, actin-GFP, stau-RBD-GFP, or an FMRP-KH-GFP fusion. Stau-RBD-GFP-expressing neurons contained less RNA in the dendrites. Scale bar: 15 μm. (C) Quantification of dendritic RNA. The RNA signal in each group was normalized to the mean RNA signal obtained for the GFP group in each dendritic segment. The analysis shows that, when compared to GFP or actin-GFP-expressing neurons, the stau-RBD-GFP dendrites had significantly less RNA in all of the dendritic compartments examined (p < 0.01). FMRP-KH-GFP-expressing neurons had RNA levels indistinguishable from either GFP or actin-GFP control neurons [N for each group are as follows: GFP (23), GFP actin (19), stau-RBD-GFP (23), FMRP-KH-GFP (10)]. (D) The decrease in dendritic RNA in stau-RBD-GFP-expressing neurons was accompanied by a significant (p < 0.01) increase in somatic RNA. This is consistent with the idea that stau-RBD-GFP binds RNA in the soma but is unable to transport it to the dendrites. (No change in somatic RNA content was observed in FMRP-KH-GFP-expressing neurons.) The increase in dendritic RNA in stau-GFP-expressing neurons (see Figure 7) was accompanied by a significant (p < 0.01) decrease in somatic RNA, suggesting that r-staufen can drive the transport of RNA to dendrites.

amount of dendritic RNA in neurons expressing stau-RBD to the amount detected in neurons expressing GFP alone or an actin-GFP fusion. As shown in Figures 5B and 5C, dendrites from RBD-expressing neurons contained significantly less RNA than the dendrites of either GFP or actin-GFP-expressing control neurons. These results suggest that stau-RBD binds RNA in the soma and prevents its transport to the dendrites. Consistent with this idea, the total somatic RNA detected in stau-RBD-expressing neurons was significantly greater than

that observed in control neurons (Figure 5D). To examine whether the reduction of dendritic RNA was specific for stau-RBD-expressing cells, we constructed an additional GFP fusion containing the RNA binding (KH) domains (Ashley et al., 1993; Siomi et al., 1993) from fragile-X-mental retardation protein (FMRP), a different RNA binding protein also present in hippocampal dendrites (Feng et al., 1997; Weiler et al., 1997). Neurons expressing FMRP-KH-GFP exhibited the fusion protein in the soma (similar to the distribution for stau-RBD-GFP) (Fig-

ure 5A). The expression of FMRP-KH-GFP, however, did not significantly alter the amount of RNA detected in hippocampal dendrites (Figures 5B and 5C), nor was the somatic RNA altered (Figure 5D).

Expression of a Truncated Staufen Reduces the Amount of Dendritic mRNA

The EtBr technique we developed potentially labels the total RNA pool, including mRNA, rRNA, and tRNA. To assess the effects of stau-RBD on the dendritic mRNA pool selectively, we used in situ hybridization to examine the distribution of poly(A) mRNA in control and stau-RBD-expressing neurons (Bassell et al., 1994; Kleiman, 1993). The antisense probe [poly(dT)] labeled mRNA in both the soma and dendrites of cultured hippocampal neurons (Figure 6A). In contrast, the signal from the sense probe [poly(dA)] was negligible. We found that neurons expressing stau-RBD contained significantly less poly(A) mRNA in their dendrites (Figures 6B and 6C) and significantly more poly(A) mRNA in their somata (Figure 6D) when compared with control neurons expressing GFP. Consistent with the EtBr results, expression of FMRP-KH domain did not reduce the poly(A) mRNA detected in the dendrites (Figures 6B and 6C). These data suggest that the RNA binding activities of FMRP might be important for local dendritic translational events, rather than RNA transport (Weiler et al., 1997). Thus, the mere overexpression of any RNA binding domain is not sufficient to decrease the dendritic RNA content; in these experiments, stau-RBD, but not FMRP-KH, reduced the dendritic mRNA pool. Furthermore, the observation that stau-RBD decreased dendritic mRNA levels but increased somatic mRNA levels argues strongly against the idea that stau-RBD is affecting mRNA stability or interfering with the antisense probe binding. Taken together with the results of EtBr labeling of the RNA pool, these data suggest that staufen is important for the delivery of RNA to the dendrites.

Overexpression of Wild-Type Staufen Increases Dendritic RNA and Changes Its Patterning

If r-staufen is essential for dendritic RNA targeting, then increasing the availability of full-length r-staufen protein might be expected to alter dendritic RNA levels. To test this hypothesis, we analyzed the dendritic RNA distribution in neurons overexpressing wild-type rat staufen fused with GFP (stau-GFP). In contrast to the reduced dendritic RNA observed in neurons expressing stau-RBD, neurons overexpressing full-length r-staufen contained more total RNA (Figures 7A and 7B) and more mRNA (Figures 6B and 6C) in their dendrites when compared to GFP or GFP-actin control cells. If the increased availability of r-staufen leads to more trafficking of RNA to dendrites, then one might expect to observe a depletion of RNA in the cell body. Indeed, this was the case: the stau-GFP-induced increase in dendritic RNA was accompanied by a significant decrease in somatic RNA and mRNA (Figures 5 and 6).

As indicated above (Figure 7), the dendritic expression pattern of stau-GFP was particularly clustered and punctate. If r-staufen is involved in the trafficking of RNAs to dendrites, then alterations in the distribution pattern of r-staufen might be expected to result in correspond-

ing changes in the distribution pattern of RNAs. We thus examined whether overexpression of r-staufen, resulting in a more robust clustering of the r-staufen protein, yielded a corresponding increased clustering of the RNA detected in dendrites. A comparison of the RNA distribution patterns in control (GFP or actin-GFP) versus stau-GFP-expressing neurons revealed that expression of stau-GFP dramatically increased the clustering of dendritic RNA (Figure 7A insets; Figure 8A). Again, we observed that the RNA distribution coincided with that of stau-GFP. In this case, the actin-GFP is particularly useful control because the distribution pattern of this protein is similar to the punctate, clustered distribution of stau-GFP. Despite this similarity in protein distribution, only stau-GFP significantly altered the RNA patterning. The coefficients of variation calculated for stau-GFP-expressing dendrites were significantly greater than either set of control dendrites, indicating that the RNA signal in stau-GFP dendrites was more variable (e.g., peaky) (Figure 8B). These data show that the specific pattern of r-staufen localization in dendrites is paralleled by a similar pattern of RNA distribution. Our data suggest that r-staufen is instrumental in determining the localization pattern of dendritic RNA.

The above idea is further supported by an experiment in which r-staufen's localization was changed from clustered to diffuse, by creating a stau-tau-GFP fusion (Figure 8C). (This fusion protein was created with the original intention of achieving axonal targeting of r-staufen. When the actual localization was examined, however, the stau-tau fusion protein was present mainly in dendrites rather than axons.) When stau-tau-GFP was expressed in cultured neurons, the protein's distribution was distinct from stau-GFP; namely, rather than being clustered, stau-tau-GFP protein appeared diffusely distributed in dendrites, perhaps consistent with tau's association with microtubules (Goedert et al., 1991). We then examined the RNA distribution in stau-tau-GFP neurons and found its distribution was also changed to a diffuse pattern (Figures 8A and 8C). Taken together with the stau-GFP overexpression data, these experiments show that there is a strong link between the localization of r-staufen and that of the dendritic RNA pool. Two different manipulations that perturbed r-staufen localization (either increasing or abolishing clustering) resulted in corresponding changes in the localization of dendritic RNA.

Discussion

The rat homolog of staufen we have cloned contains the first 4 of 5 double-stranded RNA binding domains (dsRBD) found in *Drosophila* staufen (St. Johnston et al., 1992). By contrast, recently identified mouse and human homologs contain dsRBDs 2–5. Recent studies of *Drosophila* staufen indicate that dsRBDs 1, 3, and 4 bind dsRNA, whereas 2 and 5 do not (Micklem et al., 2000). All staufen isoforms in the database appear to have an insert in RBD 2 (Micklem et al., 2000); this is also true of rat staufen. In *Drosophila* staufen, dsRBD 5 appears to be important for the actin-dependent localization (Broadus and Doe, 1997) of *prospero* mRNA (Schuldt et al., 1998; Shen et al., 1998) as well as for
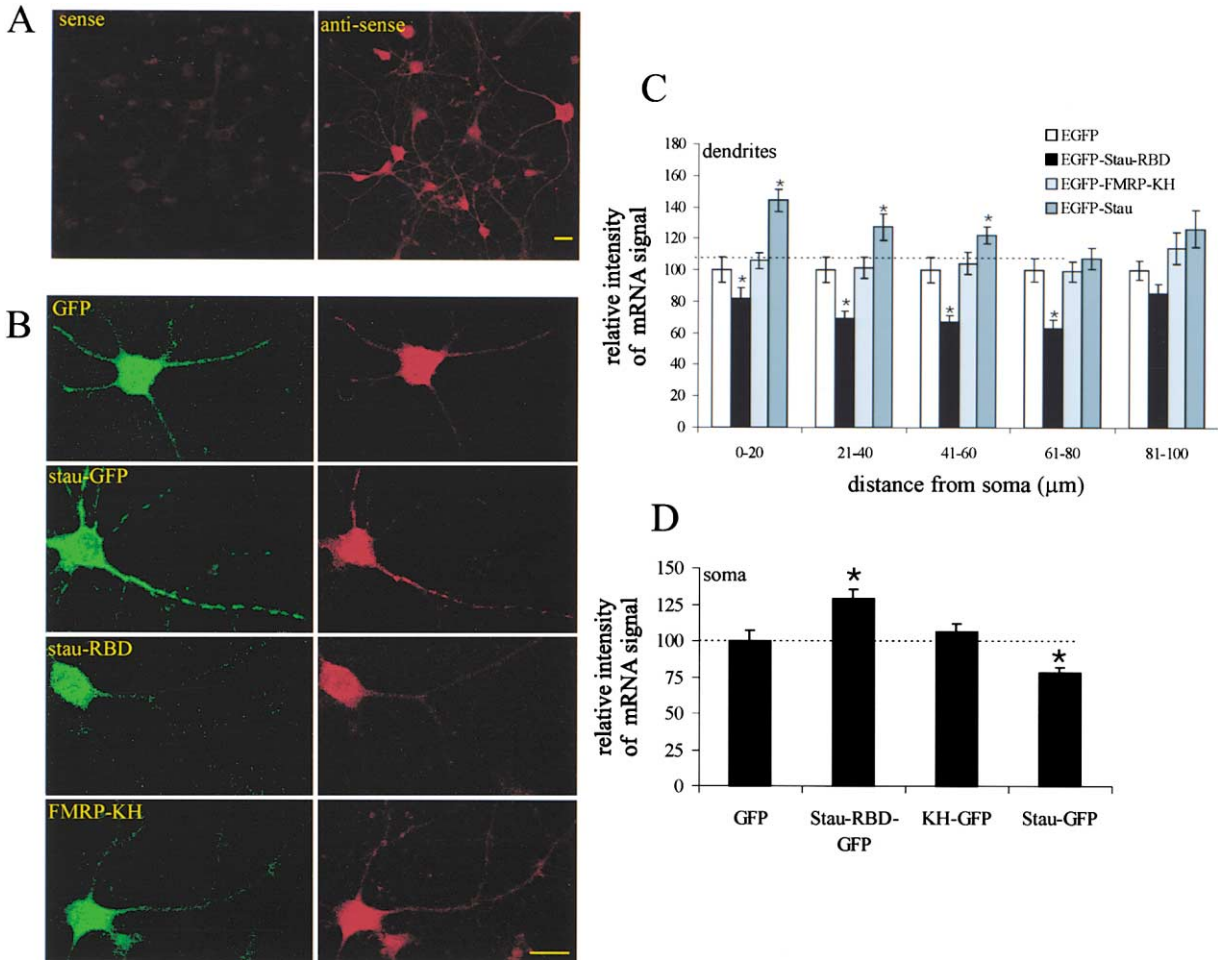
Figure 6. Expression of a Truncated R-Staufen Protein Reduces the Amount of mRNA in Dendrites

(A) In situ hybridization using poly(dA) (sense) and poly(dT) (antisense) probes. Signal for poly(A) mRNA, detected by the poly(dT) probe, is evident in the cell bodies and dendrites. Signal from the sense control is negligible. Scale bar = 15 µm. (B) In situ hybridization for poly(A) mRNA (red signal) in neurons expressing GFP, stau-GFP, stau-RBD-GFP, or FMRP-KH-GFP. Neurons expressing stau-RBD contained less poly(A) mRNA in their dendrites, when compared to GFP, stau-GFP, or FMRP-KH-GFP-expressing neurons. Scale bar = 15 µm. (C) Quantification of dendritic mRNA as assessed by in situ hybridization for poly(A) mRNA. The mRNA signal in each group was normalized to the mean mRNA signal obtained for the GFP group in each dendritic segment. The analysis shows that, when compared to GFP or actin-GFP-expressing neurons, the stau-RBD-GFP dendrites had significantly less mRNA in nearly all of the dendritic compartments examined (p < 0.05). FMRP-KH-GFP-expressing neurons had mRNA levels indistinguishable from either GFP or actin-GFP control neurons. Stau-GFP-expressing neurons had significantly more mRNA (p < 0.05) in most of the dendritic compartments examined. [N for each group are as follows: GFP (24), stau-RBD-GFP (25), FMRP-KH-GFP (27), stau-GFP (25)]. (D) The decrease in dendritic mRNA in stau-RBD-GFP-expressing neurons was accompanied by a significant (p < 0.01) increase in somatic RNA. This is consistent with the idea that stau-RBD-GFP binds mRNA in the soma but is unable to transport it to the dendrites. The increase in dendritic mRNA in stau-GFP-expressing neurons was accompanied by a significant (p < 0.01) decrease in somatic mRNA.

derepression of *oskar* translation (Micklem et al., 2000). Our rat staufen contains a truncated version of RBD 5.

Both immunostaining and expression of a staufen-GFP fusion protein revealed that rat staufen is present in both the somata and dendrites of hippocampal neurons. Staufen's distribution in dendrites was blocked by inhibition of microtubule function. This finding is similar to observations of *Drosophila* staufen, where disruption of microtubules, but not actin microfilaments, results in mislocalization of the protein (Ferrandon et al., 1994). The blockade of staufen's dendritic localization by nocodazole is also consistent with the association of a mouse staufen with tubulin in vitro (Wickham et al., 1999), the colocalization of a mammalian staufen with neuronal microtubules (Kiebler et al., 1999), and the microtubule-

dependent transport of a human-staufen-GFP fusion protein in hippocampal neurons (Kohrmann et al., 1999).

The strong coincidence of r-staufen and RNA localization was detected by simultaneous immuno- and EtBr labeling. A similar colocalization of a different staufen isoform has been observed using SYTO dyes to label RNA (Kiebler et al., 1999). In our experiments, the coincidence of the RNA and r-staufen signals was maintained when the clustering of r-staufen was either increased or decreased, further supporting the case for colocalization.

The expression of a truncated version of r-staufen (stau-RBD), which contained the RBDs but lacked the C terminus, was localized exclusively in the soma and very proximal dendrites. This stau-RBD also reduced
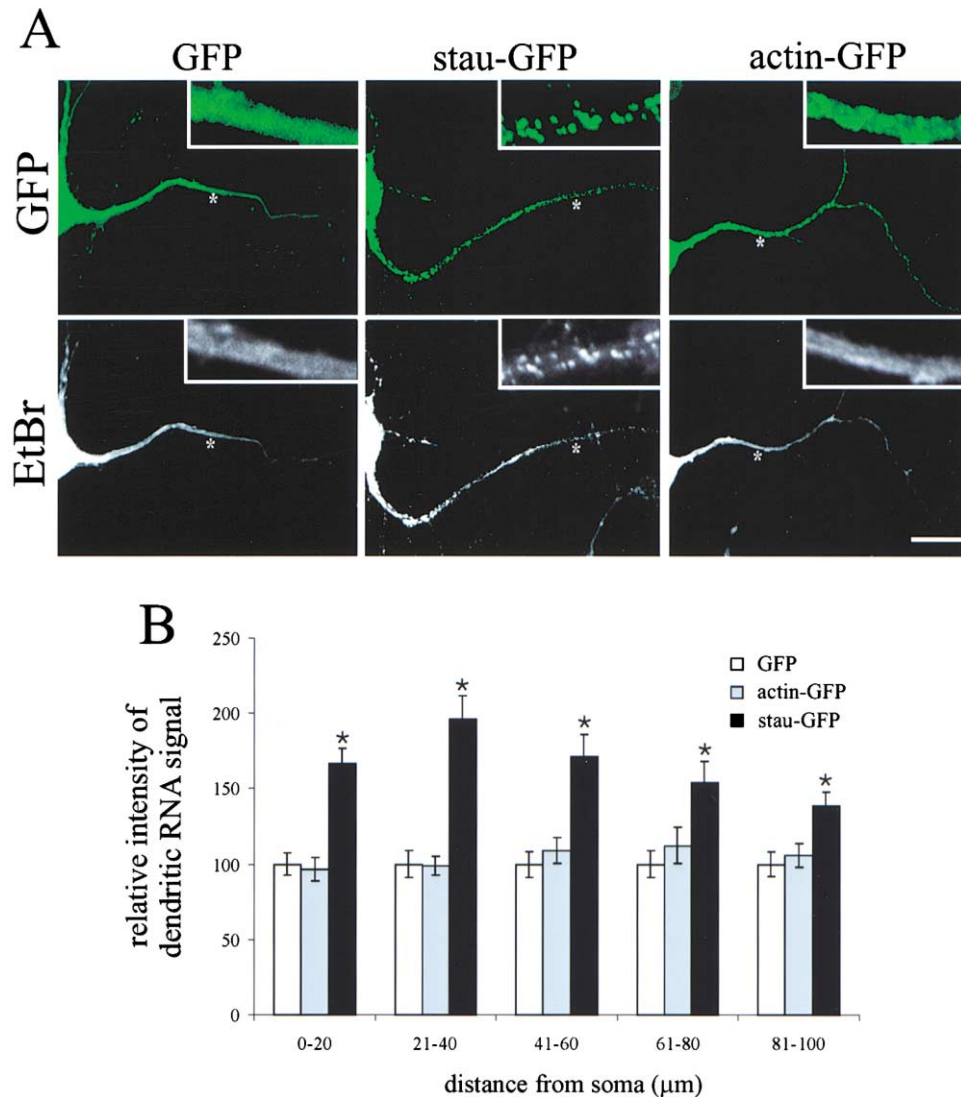
Figure 7. Expression of Full-Length R-Staufen Increases Dendritic RNA Content

(A) Shown are both the GFP and EtBr signal for three different representative neurons expressing either GFP, stau-GFP, or an actin-GFP fusion. Stau-GFP-expressing neurons contained more RNA in the dendrites than either GFP- or GFP-actin-expressing neurons. Asterisk indicates area expanded in each inset. Scale bar: 15 μm. (B) Quantification of dendritic RNA. The RNA signal in each group was normalized to the mean RNA signal obtained for the GFP group in each dendritic segment. The analysis shows that the stau-GFP dendrites had significantly more RNA in all of the dendritic compartments examined (p < 0.01). [N for each group are as follows: GFP (24), GFP actin (22), stau-GFP (23)].

the ability of full-length staufen to associate with mRNA. Using two different techniques, either EtBr labeling or in situ hybridization for poly(A) mRNA, we found that stau-RBD reduced the amount of total RNA or mRNA, respectively, detected in hippocampal dendrites. In contrast, expression of the RNA binding domain from FMRP was without effect on dendritic RNA or mRNA levels. The reduction of dendritic RNA by stau-RBD that we observed in intact neurons is complemented by a recent report of an isoform of murine staufen with a "disabled" RBD; in COS cells, overexpression of this isoform can reduce the RNA content of staufen complexes isolated biochemically (Duchaine et al., 2000).

When magnitude of inhibition produced by stau-RBD on the dendritic total RNA versus mRNA pool was compared, there was no significant difference. The similar inhibition of total RNA versus mRNA populations may

indicate that staufen acts on a common pool or target in both sets of experiments. In both *Drosophila* (Ferrandon et al., 1994) and rat (Kiebler et al., 1999), the association of staufen with ribonucleoprotein (RNP) particles has been observed. In hippocampal neurons, these granules contain individual mRNAs as well as components of ribosomes and translation regulatory factors (Knowles et al., 1996). Thus, both ribosomal RNA and mRNA may be transported to dendrites in overlapping RNP complexes. The similar inhibition observed in our EtBr and in situ hybridization experiments may therefore reflect the fact that staufen associates with this RNP complex and is necessary for its transport to the dendrites.

The ability of stau-RBD to reduce the total dendritic mRNA suggests that r-staufen associates with a potentially broad spectrum of mRNAs or, alternatively, there is
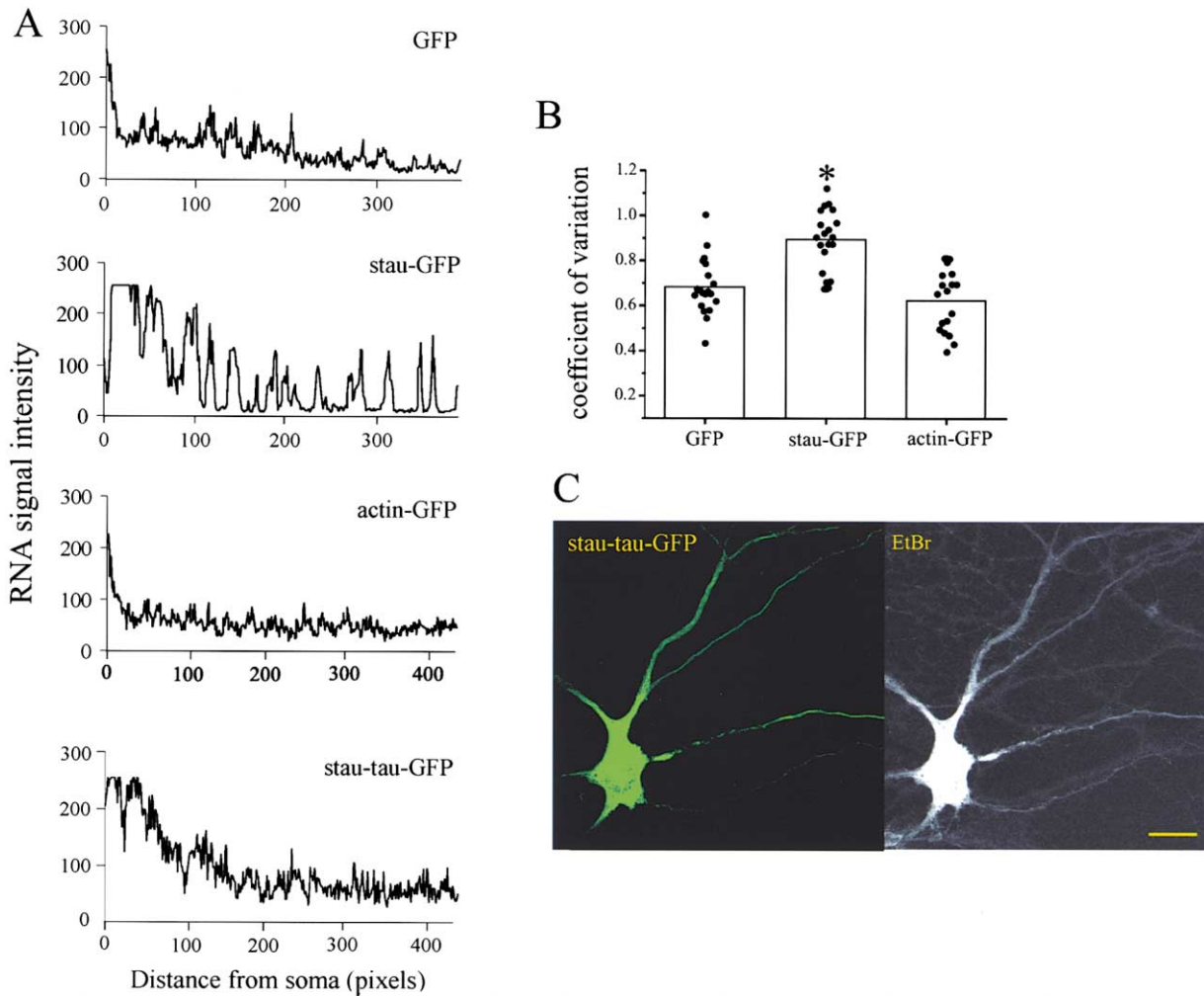
Figure 8. Alterations in Staufen's Distribution Are Accompanied by Changes in Dendritic RNA Patterning

(A) Shown are four representative examples of the linearized, digitized RNA signal obtained from dendrites of neurons expressing either GFP, stau-GFP, actin-GFP, or stau-tau-GFP. Overexpression of staufen (stau-GFP) not only increased the total amount of dendritic RNA, but also increased the clustering of RNA (also see Figure 7). (B) The coefficient of variation of RNA signals for the stau-GFP-expressing neurons was significantly greater than that of either GFP or actin-GFP neurons (p < 0.01), indicating that the RNA signal in stau-GFP dendrites was more variable. (C) The expression of a stau-tau-GFP fusion in neurons changes both the staufen and RNA distribution pattern. In contrast to the punctate distribution pattern observed for endogenous staufen (Figure 4) or stau-GFP (Figure 7A), the pattern of stau-tau-GFP was relatively homogenous throughout the dendrites. A similar, relatively homogeneous distribution of dendritic RNA was observed in these same neurons. Scale for x axis: 1 μm = 2.8 pixels.

a substantial contribution of a small number of r-staufen bound mRNAs to the total dendritic mRNA pool. Analyses of *Drosophila* staufen (Ferrandon et al., 1994), mouse-human staufen (Wickham et al., 1999), and r-staufen (DesGroseillers et al., 2001; Tang and Schuman, unpublished observations) indicate that staufen can exhibit nonselective binding for double-stranded RNA when examined in vitro or in vivo, with excessive amounts of staufen and/or the mRNA of interest present. It will be interesting to determine which mRNAs r-staufen binds in vivo, in a context with physiological concentrations of r-staufen, mRNA, and other endogenous, potentially competing, RNA binding proteins and mRNAs. If, under more physiological conditions, r-staufen still associates with multiple mRNA species, this would suggest a major

role in the transport, rather than selection, of dendritically destined RNAs.

In conclusion, our data indicate that r-staufen participates in the trafficking of mRNA to neuronal dendrites. The dendritic localization of the protein in both cultured hippocampal neurons (see also Kiebler et al., 1999; Kohrmann et al., 1999) and hippocampal slices, the active transport (see also Kohrmann et al., 1999), as well as the physical overlap with dendritic RNAs are consistent with this function. The expression of a dominant negative r-staufen reduced the dendritic mRNA pool, suggesting an active role of staufen in the delivery of mRNA to dendrites. The ability of stau-RBD to reduce the dendritic mRNA pool provides a means for establishing the importance of dendritically localized mRNAs and protein

synthesis to various forms of synaptic and behavioral plasticity. Indeed, establishing the necessity of dendritic protein synthesis for alterations in synaptic transmission and behavior remains one of the "hard" problems in this field.

Experimental Procedures

Cloning

A 460 bp EcoR1 fragment of mouse EST 533314 (Genbank Accession number AA104976) was nonradioactively labeled (ECL Random-Primed Labeling and Detection System, Amersham Pharmacia Biotech, Piscataway, NJ) and used to screen an adult rat hippocampal cDNA library (Stratagene, La Jolla, CA) per manufacturer's protocols. Of 30 positive plaques, 16 were purified and inserts were amplified by high-fidelity PCR (Roche Molecular Biochemicals Indianapolis, IN) followed by TA cloning (Invitrogen, Carlsbad, CA). Four of the isolated clones were sequenced completely and found to be overlapping partial cDNAs corresponding to r-stau. R-staufen encodes a 512 amino acid protein.

Immunohistochemistry

A GST-staufen fusion protein was generated by using the entire staufen ORF sequence. This recombinant protein was expressed and purified from bacteria, and used as the antigen for antibody induction in mice. Ascites fluid was induced and collected from the immunized animals. Immunolabeling of cultured neurons and hippocampal slices was as previously described (Tang et al., 1998).

Cultured Neurons

Dissociated hippocampal neuron cultures are prepared from postnatal day two rat pups as outlined in Banker and Goslin (1990). Neurons are plated at a density of 10,000–20,000 cells/cm^2 onto polylysine- and laminin-coated coverslips. The cultures are maintained and allowed to mature in glial-conditioned medium (Neurobasal supplemented with B27, Glutamax I, and 5 mM AraC) for seven to twenty-one days before use. In the actin or microtubule disruption experiments, inhibitors were added to medium at the same time as the infection with r-stau-GFP Sindbis virus, and incubated for 12 hr.

GFP Constructs and Sindbis Viroid Preparation

pSinRep5-EGFP-staufen: the entire r-staufen ORF sequence was PCR amplified (upstream primer: 5′cgcggatccgcgatggcaaatcccaaa gag3′; downstream promoter: 5′cgcggatccgcgtcaggcagctggggct gaactagtc3′) and cloned into the BamH1 site of pEGFP-C1 plasmid (Clontech) to create an in-frame fusion with EGFP (pEGFP-staufen). The EGFP-staufen fragment was released with NheI and XbaI (blunted) and cloned into the XbaI/StuI sites of pSinRep5 (Invitrogen). pSinRep5-EGFP-staufen-RBD: pEGFP-staufen plasmid was digested with SpeI and XbaI, blunted, and self-ligated to create the pEGFP-staufen-RBD plasmid. The EGFP-staufen-RBD fragment was released with NheI and BclI (blunted), and cloned into the XbaI/StuI sites of pSinRep5. pSinRep5-tau-EGFP- staufen: the tau coding sequence was released from pETL (Mombaerts et al., 1996) with EcoRI and SacI, blunted, and cloned into the AgeI site of pEGFP-staufen to create the ptau-EGFP-staufen. The tau-EGFP-staufen fragment was released by NheI and BclI (blunted) and cloned into the XbaI/StuI sites of pSinRep5. pSinRep5-GFP-KH: the KH domains were PCR amplified from the FMRP cDNA (Verkerk et al., 1993) using the following primers: KH5′: cgggcccgagtaagcagctggagagtt caagg3′; KH3′: cgggcccgttcaggtgataatccccaaaagaac3′. The PCR products were cloned in the ApaI site of pEGFP-C1 to create the pEGFP-KH plasmid. The EGFP-KH fusion fragment was released from pEGFP-KH by NheI/SmaI digestion and cloned into the XBAI/STUI sites of pSinRep5. Sindbis viroids were produced according to the procedures provided by Invitrogen.

RNA Binding Competition Assay and RBD
Concentration Estimation

GST-staufen and GST-stau-RBD were expressed in bacteria and purified using Glutathione Sepharose beads. The stau-RBD portion of GST-stau-RBD fusion protein was released by thrombin digestion.

For competition assays, 2 pM of GST-stau on beads was mixed with various amounts of stau-RBD protein (BSA was included to make up total protein amount of 6 pM) and ^{32}P-labeled bicoid mRNA, in an RNA binding buffer (200 mM NaCl, 10 mM TriCl, pH 8.0, 1 mM EDTA, 10% glycerol). The RNA binding reaction mixtures were incubated at 4°C for 2 hr. The GST-stau beads were then washed for 3 × 2′ with 100 μl of cold binding buffer. The bicoid mRNA present on the beads after three washes was eluted in loading buffer by boiling for 3 min, resolved on 1% agarose gel, and exposed to X-ray film. Signals after exposure were quantified using ImageJ software. A rough estimate of the relative stau-RBD concentration in infected cells was obtained by using the staufen polyclonal antibody which recognizes both stau-RBD and the full-length staufen. We compared the levels of anti-stau staining in neurons expressing stau-RBD (thus measuring together both endogenous staufen and staufen-RBD) to neurons expressing GFP (measuring only endogenous staufen). When the confocal acquisition parameters were deliberately set for low pixel intensity values (e.g., ~10) in the GFP-expressing cells, the same acquisition parameters yielded saturated (e.g., >255) signals in the stau-RBD-expressing neurons. These qualitative measurements indicated at least a 10- to 20-fold difference in concentration.

RNA Labeling and In Situ Hybridization
EtBr and Syto 14 Labeling

Hippocampal neurons (P2) cultured for 10 days were infected by Sindbis viroids for 24 hr and then fixed with 4% paraformaldehyde (containing 4% sucrose) for 20 min on ice and then with cold methanol (−20°C) for 10 min on ice. Fixed neurons were incubated with 0.2% triton-X 100 in PBS for 10 min on ice, and then stained with 1 μM of ethidium bromide in PBS for 15 min at room temperature followed by two rinses with PBS. Using this protocol, all EtBr labeling in dendrites was sensitive to RNase treatment. Prolonged exposure to EtBr at higher temperatures resulted in clear nuclear labeling that was only partially sensitive to RNase treatment, the remainder being sensitive to DNase treatment (data not shown). The stained neurons were mounted and imaged by confocal microscopy. Syto 14 labeling was conducted according to published protocols (Molecular Probes; Knowles et al., 1996). In situ probe preparation. Synthetic oligo (dT) and oligo (dA) (60 nucleotides) were 3′ end labeled with digoxigenin-11-dUTP (Roche) according to manufacturer's instruction. Probes were purified using G-50 Micro Columns (Amersham Pharmacia) followed by ethanol precipitation. The amount of purified probes was determined by measuring their optical density. The final concentration of probes in the hybridization solution was 250 ng/ml. Riboprobes for poly(A) RNA were also generated using a plasmid containing 80 bp of A/T (Kleiman, 1993), and they gave the same results as the oligo probes.

In Situ Hybridization

For hybridization, cells were fixed (4% paraformaldehyde; 4% sucrose) for 18 min at room temperature. The cells were washed with PBS/5 mM MgCl$_2$ for 3 × 5′ and 2 × SSC for 2 × 5′, followed by incubation with equilibration solution (50% formamide, 0.6 M NaCl, 10 mM Tri-HCl [pH 7.5], 1 mM EDTA, 50 μg/ml heparin, 10 mM DTT) for 5′ and with prehybridization solution (same solution containing 10% PEG 8000 and 1 × Denhardt's solution) for 2 hr at 50°C. The cells were then incubated in hybridization solution (prehyb. sol. containing 0.5 mg/ml ssDNA, 0.5 mg/ml tRNA, and 250 ng/ml probe) at 50°C overnight. After hybridization, the cells were incubated in 0.2 × SSC for 10′ followed by 2 × 30′ at 50°C, and then in PBT (1 × PBS, 2 mg/ml BSA, 0.1% Triton X-100) at room temperature for 2 × 15′. Probes were detected by indirect immunostaining using an anti-DIG monoclonal primary antibody and Cy3-conjugated secondary antibody (Jackson). After hybridization, neurons expressing EGFP or its fusion proteins were detected using anti-GFP antibody and FITC-conjugated secondary antibody.

Confocal Data Acquisition

Confocal images were acquired in 1 μm sections; image analysis was conducted on image z-stacks which contained the entire neuron of interest. EGFP and ethidium bromide were excited at 488 nm. Emitted light collected between 510–550 nm corresponds to EGFP fluorescence, whereas emitted light collected above 600 nm

corresponds to the ethidium bromide fluorescence. Cells and dendrites were chosen for analysis on the basis of their GFP expression. The experimenter did not have any knowledge of the RNA or mRNA pattern when choosing a neuron and dendrite for analysis. In addition, a subset of the data was analyzed by an experimenter blind to the experimental condition. The results of the blind analysis did not differ significantly from the rest of the analysis. In dendritic RNA quantification experiments, images were acquired with parameters that maximized the dynamic range of pixel intensity for the dendritic signal. Using these parameters, the cell body fluorescence intensity was necessarily saturated. Different acquisition parameters, which prevented saturation, were used when the cell body fluorescence was analyzed. In all experiments, identical acquisition parameters and settings were used for both control and experimental dendrites (or cell bodies) on a given experimental day. In all experiments, there was no crosstalk between the RNA and GFP signals. To analyze RNA content of individual dendrites, we binned the dendritic branch of interest into 20 μm compartments, and calculated the total pixel intensity for each compartment (NIH Image). For somatic RNA measurements, the mean RNA signal intensity was measured in the cell body (NIH Image). Group means were established for each compartment. The group means were then expressed relative to the mean pixel intensity observed in the GFP group for each compartment. Student's t tests were performed to assess statistical differences between groups. The coefficient of variation was calculated for each dendrite by determining both the mean and standard deviation of the RNA signal intensity. This statistic normalizes the data for differences in the mean, and thus accurately describes the variability of the data. We chose for analysis a single main dendrite from each neuron in each group. Thus, "N" refers to both the number of dendrites and the number of neurons per group.

References

Ashley, C.T., Wilkonson, K.D., Reines, D., and Warren, S.T. (1993). FMR1 protein: conserved RNP family domains and selective RNA binding. Science 262, 563–566.

Banker, G., and Goslin, K. (1990). Culturing Nerve Cells (Cambridge, MA: MIT Press).

Bassell, G., Singer, R.H., and Kosik, K. (1994). Association of poly(A) mRNA with microtubules in cultured neurons. Neuron 12, 571–582.

Broadus, J., and Doe, C. (1997). Extrinsic cues, intrinsic cues and microfilaments regulate asymmetric protein localization in Drosophila. Curr. Biol. 7, 827–835.

Burgin, K.E., Waxham, M.N., Rickling, S., Westgate, S.A., Mobley, W.C., and Kelly, P.T. (1990). In situ hybridization histochemisty of Ca/calmodulin dependent protein kinase in developing rat brain. J. Neurosci. 10, 1788–1789.

Casadio, A., Martin, K.C., Giustetto, M., Zhu, H., Chen, M., Bartsch, D., Bailey, C.H., and Kandel, E.R. (1999). A transient, neuron-wide form of CREB-mediated long-term faciliation can be stablized at specific synapses by local protein synthesis. Cell 99, 221–237.

Cohen, A.S., and Abraham, W.C. (1996). Facilitation of long-term potentiation by prior activation of metabotropic glutamate receptors. J. Neurophysiol. 76, 953–962.

Cohen, A.S., Raymond, C.R., and Abraham, W.C. (1998). Priming of long-term potentiation induced by activation of metabotropic glutamate receptors coupled to phospholipase C. Hippocampus 8, 160–170.

DesGroseillers, L., Kuhl, D., Richter, D., and Kindler, S. (2001). Two rat brain Staufen isoforms differentially bind RNA. J. Neurochem. 76, 155–165.

Duchaine, T., Wang, H.-J., Luo, M., Steinberg, S.V., and DesGroseillers, L. (2000). A novel murine staufen isofrom modulates the RNA content of staufen complexes. Mol. Cell. Biol. 20, 5592–5601.

Ephrussi, A., Dickinson, L.K., and Lehmann, R. (1992). oskar organizes the germ plasm and directs localization of the posterior determinant nanos. Cell 66, 37–50.

Feng, Y., Gutekunst, C.A., Eberhart, D.E., Yi, H., Warren, S.T., and Hersch, S.M. (1997). Fragile X mental retardation protein: nucleocytoplasmic shuttling and association with somatodendritic ribosomes. J. Neurosci. 17, 1539–1547.

Ferrandon, D., Elphick, L., Nusslein-Volhard, C., and St Johnston, D. (1994). Staufen protein associates with the 3′UTR of bicoid mRNA to form particles that move in a microtubule-dependent manner. Cell 79, 1221–1232.

Garner, C., Tucker, R., and Matus, A. (1988). Selective localization of messenger RNA for cytoskeletal protein MAP2 in dendrites. Nature 336, 674–677.

Goedert, M., Crowther, R.A., and Garner, C.C. (1991). Molecular characterization of microtubule-associated proteins tau and MAP2. Trends Neurosci. 14, 193–199.

Huber, K.M., Kayser, M.S., and Bear, M.F. (2000). Role for rapid dendritic protein synthesis in hippocampal mGluR-dependent long-term depression. Science 288, 1254–1256.

Kang, H., and Schuman, E.M. (1996). A requirement for local protein synthesis in neurotrophin-induced synaptic plasticity. Science 273, 1402–1406.

Kiebler, M.A., Hemraj, I., Verkade, P., Kohrmann, M., Fortes, P., Marion, R.M., Ortin, J., and Dotti, C.G. (1999). The mammalian Staufen protein localizes to the somatodendritic domain of cultured hippocampal neurons: implications for its involvement in mRNA transport. J. Neurosci. 19, 288–297.

Kleiman, R., Banker, G., and Steward, O. (1993). Subcellular distribution of rRNA and poly(A) RNA in hippocampal neurons in culture. Mol. Brain Res. 20, 305–312.

Knowles, R.B., and Kosik, K.S. (1997). Neurotrophin-3 signals redistribute RNA in neurons. Proc. Natl. Acad. Sci. USA 94, 14804–14808.

Knowles, R.B., Sabry, J.H., Martone, M.E., Deerinck, T.J., Ellisman, M.H., Bassell, G.J., and Kosik, K.S. (1996). Translocation of RNA granules in living neurons. J. Neurosci. 16, 7812–7820.

Kohrmann, M., Luo, M., Kaether, C., DesGroseiller, L., Dotti, C.G., and Kiebler, M.A. (1999). Microtubule-dependent recruitment of Staufen-green fluorecent protein into RNA-containing granules and subsequent dendritic transport in living hippocampal neurons. Mol. Biol. Cell 10, 2945–2953.

Li, P., Yang, X., Wasser, M., Cai, Y., and Chia, W. (1997). Inscuteable and staufen mediate asymmetric localization and segregation of prospero RNA during Drosophila neuroblast cell divisions. Cell 90, 437–447.

Marion, R.M., Fortes, P., Beloso, A., Dotti, C., and Ortin, J. (1999). A human sequence homology of staufen is an RNA-binding protein that is associated with polysomes and localizes to the endoplasmic reticulum. Mol. Cell. Biol. 19, 2212–2219.

Martin, K.C., Casadio, A., Zhu, H., E., Y., Rose, J.C., Chen, M., Bailey, C.H., and Kandel, E.R. (1997). Synapse-specific, long-term facilitation of Aplysia sensory to motor synapses: a function for local protein synthesis in memory storage. Cell 91, 927–938.

Micklem, D.R., Adams, J., Grunert, S., and St. Johnston, D. (2000). Distinct roles of two conserved Staufen domains in oskar mRNA localization and translation. EMBO J. 19, 1366–1377.

Mombaerts, P., Wang, F., Dulac, C., Chao, S.K., Nemes, A., Mendelsohn, M., Edmondson, J., and Axel, R. (1996). Visualizing an olfactory sensory map. Cell 87, 675–686.

Ouyang, Y., Rosenstein, A., Kreiman, G., Schuman, E.M., and Kennedy, M.B. (1999). Tetanic stimulation leads to increased accumulation of Ca(2+)/calmodulin-dependent protein kinase II via dendritic protein synthesis in hippocampal neurons. J. Neurosci. 19, 7823–7833.

Pokrywka, N.J., and Stephenson, E.C. (1991). Microtubules mediate

the localization of bicoid RNA during Drosophila oogenesis. Development *113*, 55–66.

Raymond, C.R., Thompson, V.A., Tate, W.R., and Abraham, W.C. (2000). Metabotropic glutamate receptors trigger homosynaptic protein synthesis to prolong long-term potentiation. J. Neurosci. *20*, 969–976.

Righi, M., Tongiorgi, E., and Cattaneo, A. (2000). Brain-derived neurotrophic factor (BDNF) induces dendritic targeting of BDNF and tyrosine kinase B mRNAs in hippocampal neurons through a phosphatidylinositol-3 kinase-dependent pathway. J. Neurosci. *20*, 3165–3174.

Schuldt, A.J., Adams, J.H.J., Davidson, C.M., Micklem, D.R., St Johnston, D., and Brand, A. (1998). Miranda mediates the asymetric protein and RNA localization in the developing nervous system. Genes Dev. *12*, 1847–1857.

Schuman, E.M. (1999). mRNA trafficking and local protein synthesis at the synapse. Neuron *23*, 645–648.

Sheetz, A.J., Nairn, A.C., and M., C.-P. (2000). NMDA receptor-mediated control of protein synthesis at developing synapses. Nat. Neurosci. *3*, 211–216.

Shen, C.P., Knoblich, J.A., Chan, Y.M., Jiang, M.M., Jan, L.Y., and Jan, Y.N. (1998). Miranda as a multidomain adaptor linking apically localized Inscuteable and basally localized Satufen and Prospero during asymmetric cell division in Drosophila. Genes Dev. *12*, 1837–1846.

Siomi, H., Siomi, M.C., Nussbaum, R.L., and Dreyfuss, G. (1993). The protein product of the fragile X gene, FMR1, has characteristics of an RNA binding protein. Cell *74*, 291–298.

St. Johnston, D. (1995). The intracellular localisation of messenger RNAs. Cell *81*, 161–170.

St. Johnston, D., Beuchle, D., and Nusslein-Volhard, C. (1991). Staufen, a gene required to localize maternal RNAs in the Drosophila egg. Cell *66*, 51–63.

St. Johnston, D., Brown, N.H., Gall, J.G., and Jantsch, M. (1992). A conserved double-stranded RNA-binding domain. Proc. Natl. Acad. Sci. USA *89*, 10979–10983.

Steward, O., and Levy, W.B. (1982). Preferential localization of polyribosomes under the base of dendritic spines in granule cells of the dentate gyrus. J. Neurosci. *2*, 284–291.

Steward, O., Wallace, C.S., Lyford, G.L., and Worley, P.F. (1998). Synaptic activation causes the mRNA for the IEG Arc to localize selectively near activated postsynaptic sites on dendrites. Neuron *21*, 741–751.

Tang, L., Hung, C.P., and Schuman, E.M. (1998). A role for the cadherin family of cell adhesion molecules in hippocampal long-term potentiation. Neuron *20*, 1165–1175.

Tongiorgi, E., Righi, M., and Cattaneo, A. (1997). Activity-dependent dendritic targeting of BDNF and TrkB mRNAs in hippocampal neurons. J. Neurosci. *17*, 9492–9505.

Verkerk, A.J., de Graaff, E., DeBoulle, K., Eichler, E.E., Konecki, D.S., Reyniers, E., Manca, A., Poustka, A., Willems, P.J., and Nelson, D.L., et al. (1993). Alternative splicing in the fragile X gene FMR1. Hum. Mol. Genet. *2*, 1348.

Weiler, I.J., and Greenough, W.T. (1997). Synaptic synthesis of the fragile X protein: possible involvement in synapse maturation and elimination. Am. J. Med. Genet. *83*, 248–252.

Weiler, I.J., Irwin, S.A., Klintsova, A.Y., Spencer, C.M., Brazelton, A.D., Miyashiro, K., Comery, T.A., Patel, B., Eberwine, J., and Greenough, W.T. (1997). Fragile X mental retardation protein is translated near synapses in response to neurotransmitter activation. Proc. Natl. Acad. Sci. USA *94*, 5395–9400.

Wickham, L., Duchaine, T., Luo, M., Nabi, I.R., and DesGroseillers, L. (1999). Mammalian staufen is a double-stranded RNA- and tubulin-binding protein which localizes to the rough endoplasmic reticulum. Mol. Cell. Biol. *19*, 2220–2230.

Wu, L., Wells, D., Tay, J., Mendis, D., Abbott, M.-A., Barnitt, A., Quinlan, E., Heynen, A., Fallon, J.R., and Richter, J.D. (1998). CPEB-mediated cytoplasmic polyadenylation and the regulation of experience-dependent translation of a-CAMKII mRNA at synapses. Neuron *21*, 1129–1139.

Yisraeli, J.K., Sokol, S., and Melton, D.A. (1990). A two-step model for the localization of maternal mRNA in Xenopus oocytes: involvement of microtubules and microfilaments in the translocation and anchoring of Vg1 mRNA. Development *108*, 289–298.