mRNA transport and local translation in the neuronal dendrite is implicated in the induction of synaptic plasticity. Recently, we cloned an RNA-interacting protein, SYNCRIP (heterogeneous nuclear ribonuclear protein Q1/NSAP1), that is suggested to be important for the stabilization of mRNA. We report here that SYNCRIP is a component of mRNA granules in rat hippocampal neurons. SYNCRIP was mainly found at cell bodies, but punctate expression patterns in the proximal dendrite were also seen. Time-lapse analysis in living neurons revealed that the granules labeled with fluorescent protein-tagged SYNCRIP were transported bi-directionally within the dendrite at ~0.05 μm/s. Treatment of neurons with nocodazole significantly inhibited the movement of green fluorescent protein-SYNCRIP-positive granules, indicating that the transport of SYNCRIP-containing granules is dependent on microtubules. These distributions of SYNCRIP-containing granules overlapped with that of dendritic RNAs and elongation factor 1α. SYNCRIP was also found to be co-transported with green fluorescent protein-tagged human stau1en1 and the 3' untranslated region of inositol 1,4,5-trisphosphate receptor type 1 mRNA. These results suggest that SYNCRIP is transported within the dendrite as a component of mRNA granules and raise the possibility that mRNA turnover in mRNA granules and the regulation of local protein synthesis in neuronal dendrites may involve SYNCRIP.

**Protein synthesis in neurons was long believed to occur only in the cell body, but recent evidence showing the presence of mRNA (for review, see Refs. 1–4) and the capacity for local translation of specific mRNAs in neuronal dendrites (Refs. 5 and 6; for review, see Refs. 7 and 8) has changed this belief. Selective transport and localization of certain types of mRNA and subsequent local protein synthesis in neuronal dendrites are now considered as part of the fundamental mechanisms involved in synaptic plasticity. Various kinds of mRNA, such as mRNA-coding cytoskeletal proteins (MAP2, β-actin, Arc (activity-regulated cytoskeleton-associated protein), and neurofilament proteins), kinases (e.g., the α subunit of Ca2+/CaM kinase II (CaMKIIα)), receptors and channels (glycine receptors, glutamate receptors, and inositol 1,4,5-trisphosphate receptor type 1 (IP3R1)) have been reported to target dendrites of central nervous system neurons (for review, see Refs. 4, 8). Many of the mRNAs listed above are transported to the dendrites as a component of ribonucleoprotein complexes called mRNA granules, which were detected with fluorescent dye SYTO14 (9) and by the in situ hybridization technique (10, 11). mRNA granules contain ribosomes and other components of translational machinery (9, 12, 13) as well as various mRNA-binding proteins, including fragile X mental retardation protein (14), stau1en1 (15), testis-brain RNA-binding protein (16), zip code-binding protein 1 (17), and heterogeneous nuclear ribonuclear protein (hnRNP) A2 (18). These mRNA-binding proteins are thought to be responsible for the stability and the translational regulation of mRNAs; however, their actual function in dendrites is poorly understood.**

We recently discovered a novel RNA-interacting protein, SYNCRIP (synaptotagmin binding, cytoplasmic RNA-interacting protein (19)) in mouse. A human homolog of SYNCRIP was termed as NSAP1 (20) or hnRNP Q1 (21). SYNCRIP is one of three alternative splicing variants (21) and has high homology to hnRNP R. Interestingly, in contrast to hnRNP R and other splicing variants of SYNCRIP (hnRNP Q2 and Q3), SYNCRIP is distributed throughout the cytosol instead of being localized in the nucleus (19). SYNCRIP binds to RNA in vitro, preferen-
DNAs by a standard calcium phosphate method (33) or 2.5 M glutamine (Nacalai Tesque), primers prepared from hippocampi of 1-day-old Wistar rats by a standard method to the multicloning site was removed. Sequences (MS2bs) and the coding sequence of alkaline phosphatase were upstream of which 12 copies of MS2 phage coat protein binding sequences containing IP3R1 mRNA transported within the dendrites in a study we performed a proteomic analysis of the protein component of protein complexes that stabilize c-fos proto-oncogene fos mRNA in mammalian culture cells (24), the physiological role of SYNCRIP in the cytoplasm is not yet understood. In this study we performed a proteomic analysis of the protein complex that associates with SYNCRIP in human kidney cell line 293EBNA and found that SYNCRIP preferentially associated with ribosomal proteins and RNA-binding proteins. We also found that SYNCRIP is a component of mRNA granules containing IP3R1 mRNA transported within the dendrites in a microtubule-dependent manner.

**EXPERIMENTAL PROCEDURES**

**Proteomic Analysis**—Proteomics analysis of the proteins that associate with SYNCRIP was performed as previously described (25–27). In brief, human 293EBNA cells were harvested, washed with phosphate-buffered saline (PBS), and lysed at 24 h after the transfection of CDNA coding FLAG-tagged SYNCRIP. Then the resulting cell lysate was incubated with M2-agarose overnight at 4 °C for immunoprecipitation. The protein-bound agarose beads were washed extensively, and the proteins were eluted with FLA-I (Promega). The isolated complex was precipitated using mixed methanol and chloroform. After vacuum-drying, the precipitate was digested with *Achromobacter* protease I. The digested peptide mixture were analyzed using a Direct nano flow LC-MSM system as described (26), and protein identification was performed according to the criteria described previously (26).

**Construction of Fusion Proteins**—FLAG-tagged SYNCRIP was generated by subcloning PCR-amplified DNA fragment coding mouse SYNCRIP (19) fused with FLAG tag to its N terminus into pcDNA3 (Invitrogen). To construct a green fluorescence protein (GFP)-tagged SYNCRIP, the coding sequence of mouse SYNCRIP was subcloned into the Pite/KpnI site of pEGFP-C3 (Clontech, Palo Alto, CA). Monomeric red fluorescence protein (mRFP) tag SYNCRIP (mRFP-SYNCRIP) was generated by fusing a mRFP, a gift from Dr. R. Tsien (28), to the N terminus of SYNCRIP with the amino acids Glu-Phe as a linker, which was then subcloned into pcDNA3.1/Zeo (+) (Invitrogen). To construct GFP-fused human staufen1 (GFP-hStaun1), the coding region of hStaun1 was amplified from HEp21160 (a gift from Dr. S. Sugano) and subcloned into the Xhol/HindIII site of pEGFP-C1 (Clontech).

NLS-MS2-Venus was generated from pGA14-MS2-GFP (a gift from Dr. R. Singer (29)) and pCS2-Venus (a gift from Dr. A. Miyawaki). A coding sequence of "Venus" (a variant of yellow fluorescent protein (30)) was amplified using 5'-GTGCGGCCGCTGGTATGAGCAAGGGC-GAG-G-3' and 5'-CTTGAACCTTTACTTGACAG3-'.

To construct the RNA expression vector of the 3'-untranslated regions (3'-UTR) of IP3R1 (IP3R1 3'-UTR-MS2bs), we amplified a CDNA fragment of 3'-UTR of mouse IP3R1 (bases 8579–9041) by PCR using primers 5'-GCTGCGCCGCTGGTATGAGCAAGGGC-GAG-G-3' and 5'-GCTGCGCCGCTGGTATGAGCAAGGGC-GAG-G-3'.

Time-lapse Imaging and Data Analysis—The culture medium was supplemented with 20 mM HEPES (pH 7.3) for the time-lapse imaging experiments. The temperature was maintained at ~37 °C by a heating chamber that surrounded the microscope stage. For single color time-lapse imaging, the cells were visualized under an inverted microscope (IX70; Olympus) with a 60× objective (NA 1.4; Olympus) equipped with a motorized fluorescence mirror unit exchanger, standard filter sets, and a mercury lamp. Sequential images were acquired using 3×3 spatial convolutions, where the value of each pixel is replaced with the weighted average of its 3×3 neighborhood. Center pixels are 4-fold weighted over surrounding pixels.

**Cell Culture and Transfection**—Primary cultures of neurons were prepared from hippocampi of 1-day-old Wistar rats by a standard method as described previously (31, 32) and plated on poly-l-lysine (Nacalai Tesque, Kyoto, Japan)-coated coverslips at a density of 4.6 × 10^5 cells/cm². Cells were cultured in Neurobasal medium (Invitrogen) supplemented with 2.5 mM t-glutamine (Nacalai Tesque), 2.5% (v/v) B-27 (Invitrogen), and antibiotics (250 units/ml penicillin and 250 μg/ml streptomycin). The cultures were transfected with 10 μg of DNA/b as a standard calcium phosphate method (33) or 0.5 μg of DNAs by lipofection using Lipofectamine 2000 (Invitrogen) (34) on days 5–6 in vitro. For the labeling of 3'-UTR of IP3R1 mRNA, 0.6 μg of NLS-MS2-Venus and 1.8 μg of IP3R1 3'-UTR-MS2bs were co-transfected by lipofection. The transfected cells were used for immunohistochemistry or imaging experiments 2–3 days after the transfection, which corresponded to days 7–9 in vitro.

**Antibodies**—The rabbit antibody recognizing the N-terminal region of SYNCRIP, anti-SYNCRIP-N antibody, was obtained as described previously (19). Anti-SYNCRIP-N was used after affinity purification, and the specificity of this antibody has been confirmed by parallel experiments using preimmune serum or anti-SYNCRIP-N antibody preincubated with an excess amount of antigenic polypeptide (19). Anti-elongation factor 1α (EF1α) antibody was from Upstate Biotechnology (Charlotteville, VA, clone C82-KK1). Alexa 488- or Alexa 594-conjugated secondary antibody was from Molecular Probes (Eugene, OR).

**Immunohistochemistry, Cell Labeling Experiments, and Confocal Imaging**—For immunohistochemistry of native hippocampus, Wistar rats were deeply anesthetized on postnatal day 7 and perfused with 4% paraformaldehyde in PBS. Whole brains were removed and post-fixed in the same fixative overnight at 4 °C. Sagittal sections (thickness, 100 μm) were cut with a Vibratome-type micro slicer (DTR-1500; Dosaka EM, Kyoto, Japan), collected in PBS, and permeabilized with 0.3% Triton X-100. After incubation with blocking solution (1% BSA and 0.3% Triton X-100 in PBS) sections were incubated with anti-SYNCRIP-N antibody (1:1000 dilution) in blocking solution overnight at 4 °C and subsequently with Alexa 488-conjugated anti rabbit IgG (Molecular Probes) for 3 h at room temperature. Finally, sections were mounted on slides with Vectashield (Vector Laboratories, Burlingame, CA) and observed under a confocal-scanning microscope (FV-300; Olympus) with a ×10 objective (NA 0.30; Olympus) and a ×40 objective (NA 0.85; Olympus).

For immunostaining of cultured neurons, cells were fixed with 4% formaldehyde in PBS for 10 min. After permeabilization with 0.1% Triton X-100 in PBS for 10 min and blocking with 5% skim milk in PBS. the cells were incubated with the primary antibodies at 1:1000 dilution for 3 h at room temperature. Anti-SYNCRIP-N antibody was used after affinity purification, Alexa 488- or Alexa 594-conjugated IgGs (Molecular Probes) were used as secondary antibodies. RNA labeling with ethidium bromide (EtBr) was performed as described previously (35). RNase treatment was conducted by incubating neurons with 20 μg/ml RNase A (Nippongene, Tokyo, Japan) for 15 min after the EtBr staining. For labeling hippocampal neurons with endosomal and lysosomal markers, we used Texas Red-conjugated lipofectamine in Texas Red Red-dye (Molecular Probes) overnight. Fluorescence images of cultured neurons were taken under a confocal-scanning microscope (FV-300) using a 60× objective (NA 1.4; Olympus).

All of the images taken by the confocal microscope were digitally smoothed to reduce the noise level. The smoothing filter was implemented using 3×3 spatial convolutions, where the value of each pixel is replaced with the weighted average of its 3×3 neighborhood. Center pixels are 4-fold weighted over surrounding pixels.

**Drug Preparation and Drug Treatment**—Stock solutions of nocodazole (10 mg/ml, Sigma) and latrunculin A (1 mg/ml, Molecular Probes) were prepared in dimethyl sulfoxide (Me2SO) and stored at −20 °C. Neurons were incubated with nocodazole (30 μg/ml) and latrunculin A (1 μg/ml) for 1 h at 37 °C in 5% CO2. To confirm that the cytoskeleton was disrupted, fixed cells were stained with anti-tubulin antibody (Lab Vision, CA) or Alexa 594-phalloidin (Molecular Probes). We found no
changes in the microtubules or actin structure from control cells exposed to 0.1–0.3% Me2SO (data not shown).

RESULTS

Proteomic Analysis of SYNCRIP-associated Proteins—Proteins associated with SYNCRIP were isolated by immunoprecipitation from human 293EBNA cells expressing FLAG-tagged SYNCRIP by using anti-FLAG antibody. The 111 proteins listed in supplemental Table SI were identified as components of protein complexes containing FLAG-tagged SYNCRIP. Among these 111 proteins, 44 were identified as ribosomal proteins (39.6%) and 26 were mitochondrial ribosomal proteins (23.4%), which are encoded by nuclear genes and synthesized in the cytosol (36) (Fig. 1 and supplemental Table SI). In addition, 26 RNA-binding proteins (23.4%) including 8 hnRNPs (7.2%), 4 DEAD box helicases (3.6%), and 4 splicing factors (3.6%) were also identified as SYNCRIP-associated proteins. These results suggest that SYNCRIP preferentially associates with protein complexes involved in mRNA processing and translation.

The Distribution and Dynamics of SYNCRIP in Hippocampal Neurons—mRNA granules, the ribonucleoprotein complexes present in neuronal dendrites, have been reported to contain ribosomes and other components of translation machinery (9, 12, 13) as well as a number of RNA-binding proteins (14–18). The above results of proteomic analysis in 293EBNA cells indicate that SYNCRIP preferentially associates with the major component of mRNA granules, that is, ribosomal proteins and RNA-binding proteins (Fig. 1, supplemental Table SI). In addition, SYNCRIP itself has the ability to bind to RNA, preferentially to poly(A) and poly(U) sequences in vitro (19, 22, 23). These findings led us to hypothesize that SYNCRIP is a component of the mRNA granules in neuronal dendrites.

To test this hypothesis, we investigated the expression pattern and dynamics of SYNCRIP in the dendrites of rat hippocampal neurons. Expression of SYNCRIP within the hippocampus of rats was investigated by immunohistochemistry using anti-SYNCRIP-N antibodies. SYNCRIP was expressed in the pyramidal cell layer and granular cell layer (Fig. 2A), and SYNCRIP signals were mainly observed in the cell bodies of hippocampal neurons (Fig. 2B). In addition, the proximal dendrites of pyramidal cells were also labeled with the SYNCRIP antibody (Fig. 2B, arrowheads). To investigate the localization and dynamics of SYNCRIP in detail, we transfected cultured rat hippocampal neurons with plasmid DNAs encoding mouse SYNCRIP (19) tagged with GFP (GFP-SYNCRIP). To confirm that GFP-SYNCRIP reflects the localization of endogenous SYNCRIP in cultured hippocampal neurons, we compared the immunocytochemical patterns of endogenous SYNCRIP and the distribution of GFP-SYNCRIP signals. Endogenous SYNCRIP was distributed in the dendrites as well as in the cell body of cultured hippocampal neurons (Fig. 3A, left) as was observed in native tissue. SYNCRIP was found in granules of various sizes in the dendrites (Fig. 3A, right, arrowheads). GFP-SYNCRIP also exhibited a distribution pattern very similar to that of endogenous SYNCRIP (Fig. 3B), indicating that the expression pattern of GFP-SYNCRIP reliably reflects that of endogenous SYNCRIP in cultured neurons.

Time-lapse microscopy with a CCD camera revealed that some of the granules labeled with GFP-SYNCRIP traveled within the dendrites (Fig. 4A and supplemental Movie 1). Granules labeled with GFP-SYNCRIP moved along the dendrite in both anterograde (to the periphery) and retrograde (to the soma) directions, and a change in direction of movement was observed in some granules (Fig. 4B). Double labeling of GFP-SYNCRIP with Texas Red-dextran, which is a marker for endosomes and lysosomes, indicated that the mobile GFP-SYNCRIP-positive granules were not part of the endosome-lysosomal system because GFP-SYNCRIP did not overlap with the Texas Red-dextran signal in either the cell bodies or the dendrites (data not shown). The velocity profile of the GFP-SYN-
The average velocity of the vesicle movements was about 0.05 μm/s in both the anterograde and retrograde directions as shown in Table I, and the maximum velocity of GFP-SYNCRIP movement was 0.37 μm/s. Interestingly, the speed of SYNCRIP movement was similar to that reported for mRNA granule movement (0.1 μm/s (9); 0.1 μm/s (37); 0.03–0.05 μm/s (38)).

The movements of mRNA granules have been reported to be mainly dependent on microtubules (9, 18, 35, 37–39). To determine whether microtubules are also involved in the movement of SYNCRIP-positive granules, we tested the effects of drugs that disrupt these cytoskeletal components on the velocity of granule movement. After confirming that these drugs were effective in cultured rat hippocampal neurons by immunocytochemical staining with anti-tubulin antibody (for microtubules) and Alexa 594-phalloidin (for actin filaments) (data not shown), nocodazole (30 μg/ml) and latrunculin A (1 μg/ml) were used to disrupt microtubules and actin filaments, respectively. Neither substance had a major effect on the distribution pattern or number of granules labeled with GFP-SYNCRIP. Latrunculin A did not have a significant effect on the velocity of GFP-SYNCRIP-positive granules in comparison with control cells (p > 0.1, t test, and Mann-Whitney U test). However, nocodazole significantly decreased the velocity of GFP-SYNCRIP-positive granules by ~70% in both directions compared with control cells (p < 0.001, t test and Mann-Whitney U test, Fig. 5 and Table I). These results suggest that the transport of SYNCRIP-positive granules is highly dependent on microtubules and that the contribution of the actin cytoskeleton is minor, which is consistent with previous reports for the transport of mRNA granules.
SYNCRIPT Was Co-localized with Dendritic RNAs and Markers of mRNA Granules—We then investigated whether the distribution of SYNCRIPT overlaps with that of RNA and mRNA granule markers in dendrites of cultured hippocampal neurons. Dendritic RNAs were labeled with EtBr as described previously (35). The EtBr signal was completely abolished by RNase treatment, indicating that it was specific for RNA (Fig. 6A, bottom). In the absence of RNase, EtBr labeled granular structures in the cell bodies and dendrites, which overlapped with the most of the SYNCRIPT-positive granules (Fig. 6A, top).

We also investigated whether GFP-SYNCRIPT was co-localized with protein components of mRNA granules, i.e. EF1α (9) and staufen (15, 40). Immunocytochemistry using anti-EF1α antibody revealed that GFP-SYNCRIPT-positive granules were co-localized with endogenous EF1α (Fig. 6B). However, we were not able to perform immunocytochemistry for staufen, because no specific antibody against rat staufen was available. Instead, we used GFP-tagged human staufen1 (GFP-hStau1), which is transported within dendrites as a component of mRNA granules (37). GFP-hStau1 was co-expressed with SYNCRIPT tagged with monomeric RFP (mRFP-SYNCRIPT), whose behavior was indistinguishable from that of GFP-SYNCRIPT (data not shown). mRFP-SYNCRIPT was co-localized with GFP-hStau1 in granules (Fig. 7A), and we confirmed that the GFP-hStau1-positive granules contained endogenous SYNCRIPT by immunocytochemistry (supplemental data S1). These results strongly indicate that SYNCRIPT is a component of the mRNA granule in neurons.

SYNCRIPT Is a Component of Moving mRNA Granules Containing the 3′-UTR of IP3R1 mRNA—Fig. 7B and supplemental Movie 2 show representative time-lapse images of a dendrite from a hippocampal neuron expressing mRFP-SYNCRIPT and GFP-hStau1. The mRFP-SYNCRIPT signal completely overlapped with that of GFP-hStau1 throughout the movement, indicating that SYNCRIPT is a component of the “moving” mRNA granules.

Finally, we investigated whether the SYNCRIPT-positive granules actually transport meaningful sets of mRNAs. The mRNA of IP3R1 is expressed in central nervous system neurons, including hippocampal neurons (41). In addition, IP3R1 mRNA has been shown to be present in the dendrites of cerebellar Purkinje cells and neocortical neurons (41, 42). Because a sequence homologous to the hnRNP A2 response element (A2RE, GCCAAGGAGCCAGAGGACTC), which is included in a subset of dendritically localized mRNAs generally transported as components of mRNA granules (18, 43), is found in the 3′-UTR of IP3R1 mRNA (GCAAATGAGGCAGAGGGACTC, bases identical to those of A2RE are underlined), it is a candidate for a component of mRNA granules. We visualized the 3′-UTR of IP3R1 mRNA in living neurons by a GFP-based mRNA labeling technique that was first reported in yeast (29) and was used later to visualize several mRNAs in neuronal dendrites (17, 38). We prepared two plasmids, one (NLS-MS2-Venus) containing the coding sequences of the single-stranded RNA phage capsid protein MS2 fused with Venus (a brighter variant of yellow fluorescent protein (30)) and an NLS. The other plasmid (IP3R1 3′-UTR-MS2bs) contained 12 repeats of the MS2 binding sequence, each of which encoded a 17-nucleotide RNA stem loop fused with the 3′-UTR of IP3R1 mRNA (GCAAGAAGAGGAGGGACTC, bases identical to those of A2RE are underlined). We then transfect these plasmids into hippocampal neurons, small bright granules were seen in the dendrites (Fig. 8A, top), whereas diffuse, not punctate staining was seen in the dendrites in a control experiment in which NLS-MS2-Venus alone was transfected (Fig. 8A, bottom).

### Table I

| Drug               | Anterograde μm/h | Retrograde μm/h |
|--------------------|-----------------|-----------------|
| No drug treatment  | 0.050 ± 0.051 (n = 66)a | 0.055 ± 0.058 (n = 75) |
| MeSO               | 0.041 ± 0.028 (n = 84) | 0.041 ± 0.025 (n = 79) |
| Latrunculin A      | 0.045 ± 0.023 (n = 85) | 0.043 ± 0.023 (n = 82) |
| Nocodazole         | 0.014 ± 0.010 (n = 90)b | 0.012 ± 0.009 (n = 89)b |

| a Values show average ± S.D. |
| b p < 0.001 (t test, Mann-Whitney U test) |
These granules were also labeled with mRFP-SYNCRIP when mRFP-SYNCRIP was further added to the co-transfected plasmids (Fig. 8B). A multicolor time-lapse study revealed that the movement of the IP<sub>3</sub>R1 3'-UTR mRNA signal coincided with that of the mRFP-SYNCRIP (Fig. 8C and supplemental Movie 3). Taken together, these findings indicate that SYNCRIP is a component of mRNA granules that at least transports IP<sub>3</sub>R1 mRNA.

**DISCUSSION**

In the proteomics study we showed that the SYNCRIP-associated complexes in 293EBNA cells contain at least 111 proteins, some of which seem to be responsible for mRNA processing and translation (Fig. 1 and supplemental Table SI). Cytoplasmic ribosomal proteins and RNA binding proteins such as hnRNP A2/B1, zip code-binding protein 1 (IGF-II mRNA-binding protein 1), and hnRNP U, which are the components of mRNA granules (17, 18, 44), were also detected as major SYNCRIP-associated proteins, and this fact raised the possibility that SYNCRIP plays roles as a component of mRNA granules in the neurons. Very recently, a screening study for RNase-sensitive granules that associate with motor protein kinesin revealed that SYNCRIP is one of the components of mRNA transporting granule (44). Moreover, proteomic and immunoelectron microscopic analyses showed that SYNCRIP is included in mRNA granules purified from rat brains. Our new lines of evidence further strengthen these reports that SYNCRIP is a component of mRNA granules. SYNCRIP were distributed in dendrites of hippocampal neurons as a granular structure (Figs. 2 and 3), and the SYNCRIP-containing granules were transported bi-directionally at a speed of ~0.05 μm/s in a microtubule-dependent manner (Fig. 4 and Table I), as was...
previously reported for mRNA granule dynamics (9, 18, 35–37, 44). SYNCRIP in the dendrite co-localized with RNA and the component of mRNA granules (Figs. 5–7) and was co-transported with IP$_R$1 mRNA (Fig. 8). The molecular mechanism underlying the recruitment of SYNCRIP into mRNA granules remains unknown. One possibility is that SYNCRIP interacts with poly(A) or AU-rich regions that are generally present in the 3' region of mRNAs, as was supported by the fact that SYNCRIP interacts directly with poly(A) and poly(U) in vitro (19, 22, 23). Another possibility is that SYNCRIP is bound to mRNA granules as a component of a protein complex as is reported for a human homolog of SYNCRIP in a non-neuronal cell (24). Clarification of the binding relations among the components of mRNA granules may be required to investigate these possibilities.

It is interesting that a number of mitochondrial ribosomal proteins associated with SYNCRIP in 293EBNA cells (Fig. 1 and supplemental Table S1). However, SYNCRIP was not found in mitochondria either in the human kidney cell line and the cultured hippocampal neuron (data not shown). SYNCRIP might associate with mitochondrial ribosomal proteins in the cytoplasm and have indirect roles in protein synthesis in mitochondria, but further study is required to test this possibility.

Microtubule-based motor proteins kinesin and dynein are possibly responsible for the transport of SYNCRIP-positive mRNA granule, since they were co-purified with SYNCRIP-containing mRNA granules (44).2 mRNA granules that contain cytoplasmic polyadenylation element-binding protein has also been shown to be co-localized with molecular motor kinesin and dynein (39). Bi-directional movement of GFP-SYNCRIP-positive granules (Fig. 4) could be explained by mixed polarity of the dendritic microtubules (45) or by coordination of multiple, opposite-directed motor proteins. In the present study, actin filaments were shown to contribute little to the transport of SYNCRIP (Fig. 5 and Table I), which has also been reported to be the case for the movement of hnRNP A2-positive mRNA granules (18). Although actin filaments seem less involved in the transport of mRNA granules, an actin motor myosin V is shown to be a component of the mRNA/protein complex containing tauen and fragile X mental retardation protein (46).

Our results do not necessarily exclude a possible association of SYNCRIP-containing mRNA granules with actin filaments.

The speed of GFP-SYNCRIP-positive granule movement (~0.05 μm/s, Fig. 5) was comparable with that of mRNA granules visualized by SYTO14 (0.1 μm/s, 9), of tauen-containing granules (~0.1 μm/s, 37), and of mRNA granules containing the 3'-UTR of CaMKII mRNA (0.03–0.05 μm/s (38)). However, the speed of GFP-SYNCRIP was much slower than that of zip code-binding protein 1 and β-actin mRNA-positive granules, which move at an average velocity of ~1.0 μm/s (17), which exceeds the maximum speed of GFP-SYNCRIP movement (0.37 μm/s). This result suggests a possibility that SYNCRIP is not involved in mRNA granules that are transported at a fast speed. Variety in the transport speeds of mRNA granules may reflect the heterogeneity in mRNA granules that has been proposed recently (47).2 In addition to the differences in the motor proteins interacting with each subset of mRNA granules, interaction between mRNA granules and organelles may modulate the dynamics of mRNA granules. mRNA granules containing tauen and fragile X mental retardation protein are reported to associate with rough endoplasmic reticulum (46), and this association may affect the transport of mRNA granules. Further work is required to understand the heterogeneity in mRNA granules and their transport.

In this study, we have demonstrated that 3'-UTR of IP$_R$1 is co-transported with SYNCRIP as mRNA granules (Fig. 8). Although IP$_R$1 mRNA was previously shown to be present in the dendrites of cerebellar Purkinje cells and neocortical neurons (41, 42), the mechanism underlying the dendritic distribution of IP$_R$1 mRNA has never been elucidated. This study suggests that IP$_R$1 mRNA may be delivered into the dendrites via mRNA granule transport and that 3'-UTR of IP$_R$1 mRNA may contain a targeting signal to dendritic mRNA granules, as is known for other dendritic localized mRNAs. IP$_R$ plays an important role, such as induction of synaptic plasticity in neuronal dendrites (48–50). The dendritic localization and local translation of CaMKII mRNA are responsible for the delivery of the kinase and for the induction of synaptic plasticity (51). The dendritically transported mRNA granules containing IP$_R$1 mRNA may also be important for an accurate delivery of IP$_R$1 proteins into the post-synapse and for the induction of synaptic plasticity.

Although the physiological roles of SYNCRIP are still poorly understood even in non-neuronal cells, some interesting properties of SYNCRIP have been reported. Because SYNCRIP is a component of a protein complex that stabilizes c-fos mRNA (24), it is possible that SYNCRIP also stabilizes mRNAs during their transport in dendrites. Insulin stimulation and osmotic shocks are reported to induce phosphorylation of tyrosine residues of SYNCRIP, and the RNA binding activity of SYNCRIP is modified by phosphorylation (22, 23). Insulin is present in the brain (for review, see Ref. 52), and insulin receptor tyrosine kinases are abundant in the hippocampus, especially in neuronal dendrites, including synapses (53, 54). In addition, overexpressed fibroblast growth factor receptor 1, a receptor tyrosine kinase that is expressed in hippocampal neurons (55), is also shown to phosphorylate a human homolog of SYNCRIP (NSAP1) on its tyrosine 373, which is located in the third RNA recognition motif domain (56). These studies raise intriguing possibilities that the turnover or translation of mRNAs contained within mRNA granules could be controlled by the insulin- or fibroblast growth factor-dependent phosphorylation of SYNCRIP in hippocampal neurons. Elucidating the functions of SYNCRIP and its regulatory mechanism in mRNA granules may provide an important key for understanding the temporal and spatial regulation of the local translation of mRNA involved in the induction of synaptic plasticity.

Acknowledgments—We thank Dr. R. Tsen (University of California, San Diego, CA) for the gift of monomeric RFP, Dr. S. Sugano (The Institute of Medical Science, The University of Tokyo, Tokyo, Japan) for the gift of a cDNA clone for human tauen (HEP22160), Dr. R. Singer (Saint Mary's University, Nova Scotia, Canada) for the gift of pGA14-MNS-23-GFP, Dr. A. Miyawaki (National Institute, McGill University, Quebec, Canada) for valuable discussions. We also thank M. Iwai (The University of Tokyo) for assistance with plasmid construction.

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