KIF4 Mediates Anterograde Translocation and Positioning of Ribosomal Constituents to Axons

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In this study, we have used a combination of biochemical and molecular biology techniques to demonstrate that the C-terminal tail domain of KIF4 directly interacts with P0, a major protein component of ribosomes. Besides, in dorsal root ganglion neurons, KIF4 and P0, as well as other ribosomal constituents, colocalize in clusters distributed along axons and neuritic tips. RNA interference suppression of KIF4 or expression of KIF4 lacking the tail domain result in accumulation of P0 and other ribosomal proteins at the cell body and in their disappearance from axons. Our results also show one additional function for KIF4 involving an Ezrin-Radixin-Moesin-like domain in the second coiled-coiled region of KIF4. Expression of a KIF4 mutant lacking this domain abolishes the clustering of ribosomal constituents and prevents the anterograde translocation of the cell adhesion molecule L1. Taken together, the present results suggest that by binding to P0 through its tail domain and by using its motor activity, KIF4 is involved in the anterograde trafficking of ribosomal constituents to axons and that by means of its Ezrin-Radixin-Moesin-like domain interacts and transports L1.

KIF4 is a 1,231-amino acid kinesin superfamily member composed of an N-terminal globular motor domain, a central helical stalk domain, and a C-terminal tail domain. KIF4 forms a homodimer that moves along microtubules toward the plus-end at a velocity of 0.2 \( \mu \text{m/s} \) (1, 2). KIF4 is predominantly expressed in juvenile tissues including developing neurons, where it associates with a population of small vesicles localized to neurites and growth cones (1, 3) that contain as one of its components the cell adhesion molecule L1 (3). KIF4 also localizes to the cell nucleus, where its C-terminal domain suppresses the activity of poly(ADP-ribose) polymerase-1, an enzyme known to maintain cell homeostasis by repairing DNA and serving as a transcriptional regulator (4). Other studies have demonstrated a role for KIF4 in mitosis (5–9), tumor formation (10), and viral trafficking (11). We now report on the association of KIF4 with P0, a major and essential protein component of ribosomes, and its involvement in the anterograde transport and positioning of ribosomal constituents to axons of developing neurons.

EXPERIMENTAL PROCEDURES

Plasmids Constructs—The cDNA for rat KIF4 was amplified by reverse transcription-PCR from mRNA isolated from the cerebral cortex of 3-day-old rats with the following primers: 5\textsuperscript{'-}ATG AAA GAA GAG GTG CAG GCT GGA GTT GTG TG-3\textsuperscript{'} and 5\textsuperscript{'-}AAT GCT GCC GTT GTC TGA GTG AGG-3\textsuperscript{'} for the N-terminal fragment of KIF4; and 5\textsuperscript{'-}AGG-3\textsuperscript{'} and 5\textsuperscript{'-}TCT GTT TGC TGG CTA CTG TTG-3\textsuperscript{'} for the C-terminal fragment of KIF4. KIF4 cDNA was then subcloned in pEGFP-C3 and in pCMV-Myc (Clontech). The KIF4-C-terminal (bp 3250–3789) was cloned using a BamHI/BamHI restriction site in pCMV-HA vector (Clontech). The N-terminal fragment of P0 (bp 1–591) was amplified by PCR from Mouse Brain MATCHMARKER cDNA Library of 9–12 weeks old (Clontech) with primers 5\textsuperscript{'-}GTC GTG GTC TGA GTG CTA CCA TTA ACT GGA AGA GTG CGG-3\textsuperscript{'} and 5\textsuperscript{'-}AAA CAC C-3\textsuperscript{'} and subcloned in HA-P0-FL. Auto rigor mutant KIF4 was generated with a site-directed mutagenesis kit (Stratagene) by changing Gln\textsuperscript{86} into Leu\textsuperscript{86} and Thr\textsuperscript{89} into Asn\textsuperscript{89}. The P0-C-terminal fragment (bp 310–955) was cloned from clone 44 using SalI/XhoI restriction sites in pCMV-HA vector (Clontech). The N-terminal fragment of P0 (bp 1–591) was amplified by PCR with the following primers: 5\textsuperscript{'-}AGG-3\textsuperscript{'} and 5\textsuperscript{'-}TCT GTT TGC TGG CTA CTG TTG-3\textsuperscript{'} for the N-terminal fragment of KIF4; and 5\textsuperscript{'-}AGG-3\textsuperscript{'} and 5\textsuperscript{'-}TCT GTT TGC TGG CTA CTG TTG-3\textsuperscript{'} for the C-terminal fragment of KIF4. KIF4 cDNA was then subcloned in pEGFP-C3 and in pCMV-Myc (Clontech). The cDNA encoding Δ-TL-KIF4-GFP (bp 1–315) was cloned in pEGFP-C3 with HindIII/HindIII restriction sites in pCMV-HA vector (Clontech). KIF4 and P0, and the anterograde and retrograde transport of ribosomal constituents to axons of developing neurons.

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\textsuperscript{4}The on-line version of this article (available at http://www.jbc.org) contains supplemental Table S1 and Figs. S1–S5.
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GTC AAA TAG CTG AGT TGA AAA GCT TTT CAA CTC AGC TAT TTG ACC-3' as targeting sequences for sh1-KIF4-GFP, and 5'-GGT GTT CCT GCA AAG GCT AAG CTT AGC TTT TGG AAC ACC CTT TTT G-3' plus 5'-AAT TCA AAA AGG GTG TTC CTG CAA AGG CTA AGC TTA GCC TTT GCA GGA AAC C-3' as targeting sequences for sh2-KIF4-GFP. The DNA fragments containing U6-KIF4-si and U6-control-si were inserted into pCAGIG vector in which the GFP-cDNA is under the control of chick actin-minimal cyto-megalovirus promoter; the resulting plasmids were referred to as sh1-KIF4-GFP and sh2-KIF4-GFP. Two scramble control-siRNA plasmids (S-sh1 and S-sh2) were purchased from Ambion Co. In addition, a third scramble control RNAi3 (S-sh3) was prepared from the sh1-KIF4-GFP; for such a purpose two nucleotide changes were made in the target sequence: GGG TCT ACT AGC TGA GTT GAA.

**Yeast Two-hybrid Screening**—Yeast two-hybrid screening was performed using the Matchmaker two-hybrid system (Clontech Laboratories, Palo Alto, CA). To construct the bait plasmid, the KIF4 tail domain (bp 3250–3738) was cloned into pGBKKT7 in frame with the GAL4 DNA-binding domain using BamHl/BamHI restriction site. The yeast strain AH109 was transformed with pGBKKT7-KIF4 tail domain and an 18-day-old embryonic mouse brain cDNA library fused with the GAL4 activation domain in the pACT2 vector. Transformants expressing both the bait and interacting prey proteins were selected on SD-Trp/-Leu/-His/-Ade and incubated at 30 °C for 5–7 days. β-Galactosidase activity was tested using the filter lift assay to identify the positive colonies. Approximately 1 × 10⁶ colonies were screened, and 21 positive clones were identified. pACT2-cDNA constructs of the positive clones were isolated, sequenced, and analyzed by comparison with the GenBank3 sequence data bank. The control plasmids pGBKKT7–53, pGBKKT7-lamC, and pGADT7-T were from Clontech.

**Preparation of KIF4 mAbs**—The last 161 amino acids of the C-terminal tail domain of rat KIF4 were used as immunogen for the production of mAbs. Briefly, BALB/c mice (University of La Plata, La Plata, Argentina) were immunized four times (days 0, 7, 14, and 21) by intraperitoneal injection of 25 μg of the immunogen in phosphate-buffered saline mixed 1:1 with an antibiotic mixture (100 μg/ml penicillin, 100 μg/ml streptomycin, and 0.25 μg/ml fungizone; Invitrogen), and 1% hypoxanthine-amino-zeptin-thimidine (HAT supplement; Sigma). The cells were plated into 96-well plates and were grown at 37 °C in a humidified atmosphere in 5% CO₂. Positive clones were detected by screening supernatants by indirect immunofluorescence and enzyme-linked immunosorbent assay. Two clones designated as mAbs 2G3 and 11D3 were then tested by Western blotting of recombinant or endogenous KIF4 (see also Ref. 11).

**Cultures, Transfection, Microinjection, and Immunofluorescence**—Dorsal root ganglia (DRG) cell cultures were prepared using standard procedures. Briefly, DRG from the cephalic, thoracic, and lumbar regions were dissected from 2–5-day-old Sprague-Dawley rats of either sex and kept at 4 °C in Hanks’ balanced salt solution until dissociated by enzymatic digestion with 1 mg/ml collagenase for 20 min and 0.25% trypsin for 10 min at 37 °C, followed by gentle mechanical dissociation using a fire polished Pasteur pipette in minimum essential medium containing 10% fetal bovine serum (MEM10). To obtain a highly purified neuronal cell population, once dissociated, the cell suspension was centrifuged at 100 × g for 1 min, and the supernatant was discarded. Resuspended cells were plated onto an uncoated plastic dish for 1 h to allow non-neuronal cells to attach to the dish. The medium was recovered, and neurons were plated at a density of 1000–1200 neurons/cm² on coverslips coated with 100 μg/ml poly-d-lysine plus 10 μg/ml laminin and a kept at 37 °C in MEM10 for 1 h. After neurons attached to the substrate, the medium was changed for Dulbecco’s modified Eagle’s medium supplemented with N2, B27, and 20 ng/ml nerve growth factor. To inhibit fibroblast proliferation, 5 mM d-arabinofuranoside cytosine was added to the culture medium. The cells were maintained in a humidified 37 °C incubator with 4% CO₂. DRG neurons or CHO cells were transfected with Lipofectamine 2000 as described (37). DRG neurons were also microinjected into the nucleus with cDNAs encoding the protein of interest with one 0.2-s pulse of ~80 hPa N2 pressure using an automated microinjection system (Eppendorf Microinjection system 5242) placed in an inverted phase contrast/differential interference contrast microscope (Carl Zeiss). The cDNAs were prepared in microinjection buffer (10 mM HEPES, 140 mM KCl, pH 7.4) and microinjected at 0.08–0.25 μg/μl depending on the plasmid, using back loaded glass capillaries and a micromanipulator (Carl Zeiss). During microinjection, the neurons were maintained in Leibovitz’s (L-15) medium to prevent pH changes at 37 °C. After microinjection, the cells were returned to Dulbecco’s modified Eagle’s medium supplemented with N2, B27, and nerve growth factor and maintained at 37 °C in a humidified CO₂ environment for 18–20 h to allow the expression of injected cDNAs. Cultured cells were fixed and processed for immunolabeling as described (32, 37). A complete list of the primary antibodies used in this study can be found as supplemental data. For some experiments, cells were extracted with detergents prior to fixation under microtubule-stabilizing conditions (32). All of the immunostained cells were analyzed by confocal microscopy using either an Olympus Fluoview1000 Spectral or Zeiss Pascal confocal microscopes.

**Subcellular Fractionation**—Ribosomal salt wash (RSW) fractions were prepared as described previously (31). Briefly, 3-day-old rat cerebral cortex was homogenized in buffer A (10 mM HEPES, pH 7.5, 1 mM potassium acetate, 1.5 mM magnesium acetate, 2 mM dithiothreitol, and protease inhibitor mixture). The homogenate was centrifuged for 10 min at 4 °C and 10,000 × g, and the supernatant fraction (low speed

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3 The abbreviations used are: RNAi, RNA interference; ERM, Ezrin-Radixin-Moesin; mAb, monoclonal antibody; DRG, dorsal root ganglia; CHO, Chinese hamster ovary; RSW, ribosomal salt wash; HA, hemagglutinin; SN, supernatant; GFP, green fluorescent protein.
supernatant) was layered onto a cushion of 30% (w/v) sucrose in buffer A and centrifuged for 3.5 h at 4 °C and 90,000 × g. The supernatant (high speed supernatant) and interphase (membrane-enriched fraction) were collected. The pellet was resuspended in buffer A at a concentration of 50–100 A260 units/ml, adjusted to 1 M NaCl, incubated for 45 min at 4 °C in an overhead rotator, and centrifuged for 45 min at 4 °C and 100,000 × g. The supernatant fraction represented the RSW fraction. All of the obtained fractions were then subjected to electrophoresis, transferred to polyvinylidene difluoride membranes, and probed with different antibodies.

Other Methods—Immunoprecipitation and immunoblotting assays were carried as described previously (3, 32, 37).

RESULTS

KIF4 Interacts with P0—The tail domain of KIF4 is supposed to serve as a cargo adaptor that associates with specific proteins (2, 4). Therefore, to identify proteins binding to KIF4, the tail domain of KIF4 (amino acids 1005–1231) was prepared as a bait to screen an 18-day-old embryonic mouse brain cDNA library in a yeast two-hybrid system. Among other hits, we identified a polypeptide (clone 44) corresponding to amino acids 103–317 of ribosomal protein P0 as a potential interacting protein. P0 is a 34-kDa (314 amino acids) component of the eukaryotic ribosome “stalk,” which is a highly flexible lateral protrusion of the large ribosomal subunit that plays a central role in the interaction of elongation factors with the ribosome during protein synthesis (12, 13). The observation that KIF4 preferentially localizes to axons of young neurons (3) and that ribosomal constituents, RNA particles, and protein synthesis have been detected in axons of developing neurons (14–26) prompted us to consider the possibility that trafficking of ribosomal constituents to axons is mediated by KIF4 via a direct interaction with P0. The binding of KIF4 to P0 was first verified by an independent pair wise yeast two-hybrid analysis on high stringency conditions. This interaction was also evaluated and confirmed by coimmunoprecipitation of cell extracts of CHO cells transfected with Myc-tagged KIF4-CT (C-terminal fragment), or Myc-tagged KIF4-FL (full length) and HA-tagged P0-CT, or HA-tagged P0-FL (Fig. 1, A–D). No such interaction was detected in cells coexpressing a mutant form of KIF4 lacking the C-terminal domain (KIF4 tail-less, Δ-CT) and P0 (Fig. 1E). This binding was specific to KIF4 because immunoprecipitation assays of cell extracts of CHO cells transfected with Myc-tagged KIF2, another kinesin superfamily member (2), and HA-tagged P0-FL (Fig. 1F) did not lead to recovery of P0 with the beads. The ability of endogenous KIF4 to interact with P0 was then assessed in microsomal fractions obtained from 3-day-old rat brain by coimmunoprecipitation with the KIF4 mAb (clone 2G3) and immunoblotting with the P0 antiserum (Fig. 1G). We then asked whether KIF4 was present in fractions containing RNA granules, structures that correspond at the ultrastructural level to a large amorphous collection of ribosomes (14). Abundant components of this fraction include ribosomal proteins (e.g. P0, P1, P2), RNA-binding proteins (e.g. Staufen), transported mRNAs (e.g. β-actin mRNA), and motors proteins, (e.g. KIF5b and dynein) (14, 27–30). Following the procedures described by Rehbein et al. (Ref. 31; see also “Experimental Procedures”), embryonic (embryonic day 18) brain tissue was subjected to subcellular fractionation, to obtain a low speed supernatant, a high speed supernatant (SN), a membrane-enriched fraction, and a RSW fraction that were analyzed by immunoblotting with mAbs against KIF4, ribosomal P0, ribosomal P1 and P2, other constituents of the ribosomal stalk, ribosomal protein S6, a protein of the small ribosomal subunit, and a marker of putative RNA granules (31), Staufen, and the cell adhesion molecule L1. As expected according to our previous subcellular fractionation study (3), KIF4 was present in the low speed supernatant, SN, and membrane-enriched fractions. Interestingly, KIF4 was also detected in the RSW fraction, where ribosomal and RNA-binding proteins were highly enriched, and membrane proteins, such as L1, were largely absent (Fig. 1H). Then we tested whether it was possible to immunoprecipitate P0 from the RSW fraction with KIF4 mAbs; the results showed that KIF4 mAb 2G3 quantitatively and significantly recovered KIF4 and P0 and that in this immunoprecipitated fraction, S6 and Staufen were also present (Fig. 1I). Similar results were obtained with mAb 11D3. No immunoprecipitation of KIF4 or P0 was detected after incubation of the RSW fraction with KIF4 preimmune serum (Fig. 1J, lower panels). Taken together, these data confirm the interaction detected by the two-hybrid screen and suggest that endogenous KIF4 forms a complex with P0 in brain tissue. We then asked whether KIF4 and P0 colocalize in cultured cells. To this end, we first analyzed the distribution of ectopically expressed GFP-KIF4 (GFP fused to the N terminus of KIF4-FL) and Myc-tagged P0 in non-neuronal flat cells (CHO cells) where the distribution of cytoskeletal elements and organelles can be easily evaluated. Fig. 1J, shows that GFP-KIF4 localizes to the cell nucleus and in punctate-like structures located along microtubules; interestingly, GFP-KIF4 positive structures were also detected in the peripheral actin-rich region of the cell (Fig. 1J, arrows). In CHO cells coexpressing P0, we detected colocalization with KIF4, which was particularly evident at the peripheral leading edge (Fig. 1, K–M). This interaction was also detected in cells extracted with detergents previous to fixation under microtubule-stabilizing conditions (32; see also “Experimental Procedures”) (Fig. 1, N–P).

KIF4 Colocalizes with P0 in DRG Neurons—In the next series of experiments we decided to determine whether KIF4 and P0, as well as other ribosomal constituents, colocalized within axons of developing neurons. Peripheral sensory neurons, such as DRG cells from embryonic, newborn, and adult rodents, have been used to study local protein synthesis under normal (23, 26) and regenerative conditions (33–35). Therefore, we first analyzed the distribution of endogenous KIF4 and P0 in DRG neurons cultured on a laminin substrate and in the presence of nerve growth factor. Within a day after plating, these neurons extend long and branched axons that stain positively for dystrokinin α-tubulin (supplemental Fig. S1), a marker of stable microtubules, and for Tau or L1, two axonal markers. Immunofluorescence staining of these neurons with the KIF4 mAb 2G3 revealed staining of the cell body and foci (clusters) of strong signal intensity along axons (supplemental Fig. S1). Ribosomal protein P0, which shows a similar distributional pattern (supplemental Fig. S1), displays extensive colocalization...
with KIF4 within axons, branch points, and growth cones (Fig. 2A and supplemental Fig. S1). Similar colocalization was detected after coexpression of Myc-tagged KIF4 or GFP-KIF4 and HA-tagged P0 (Fig. 2, B–D). By contrast, almost no colocalization of endogenous kinesin heavy chain with P0 was found in axons of DRG neurons; thus, most P0 clusters contain
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little if any kinesin heavy chain immunofluorescence (supplementary Fig. S1). To further test for colocalization of KIF4 with ribosomal constituents, we compared the distribution of GFP-KIF4 with that of Staufen and ribosomal protein S6; as expected, confocal microscopy revealed extensive colocalization of ectopically expressed KIF4 with Staufen (Fig. 2, E–G); note the colocalization of both proteins (arrows). B and C, confocal images showing colocalization of ectopically expressed GFP-KIF4 (green) and HA-tagged P0 (red, arrows). D, dark field views of a branch point short arrow and a growth cone (long arrow) of a DRG neuron showing colocalization of GFP-KIF4 and HA-tagged P0. E–G, confocal images showing the distribution of ectopically expressed GFP-KIF4 (green) and endogenous ribosomal protein S6 (red); note that many GFP-KIF4 labeled structures (arrows) are also immunoreactive for Staufen (yellow in G). H–J, confocal images showing the distribution of ectopically expressed GFP-KIF4 (green) and endogenous ribosomal protein S6 (red); note that many GFP-KIF4 labeled structures (arrows) are also immunoreactive for S6 (yellow in J).

FIGURE 2. KIF4 colocalizes with P0 in DRG neurons. A, merge confocal image showing the distribution of endogenous KIF4 (green) and P0 (red) as revealed by staining with the KIF4 mAb 2G3 and the anti-P0 polyclonal antibody. Note the colocalization of both proteins (arrows). B and C, confocal images showing colocalization of ectopically expressed GFP-KIF4 (green, arrows) and HA-tagged P0 (red, arrows). D, high power views of a branch point (short arrow) and a growth cone (long arrow) of a DRG neuron showing colocalization of GFP-KIF4 and HA-tagged P0. E–G, confocal images showing the distribution of ectopically expressed GFP-KIF4 (green) and endogenous Staufen (red); note that many GFP-KIF4 labeled structures (arrows) are also immunoreactive for Staufen (yellow in G).
RNAi suppression of KIF4 prevents the anterograde translocation of ribosomal constituents. A–D, sh1-KIF4-GFP (A) and sh2-KIF4-GFP (C) inhibit KIF4 expression as revealed by staining with the mAb 2G3 (B and D). E–G, confocal images showing expression of sh1-KIF4-GFP (green) and the distribution of tyrosinated α-tubulin (blue) and endogenous P0 (red). Note that most all P0 staining is restricted to the cell body. H–J, confocal images showing expression of control-sh-GFP (S-sh1; green) and endogenous tyrosinated α-tubulin (blue) and P0 (red). Note the presence of P0 clusters. For these experiments, DRG neurons were microinjected with the RNAi 2 h after plating and examined 24 h later. K and L, a similar experiment but showing confocal images of a DRG neuron microinjected with sh2-KIF4-GFP (green) and stained with the polyclonal antibody against Staufen (red). Note the reduction of Staufen staining along neurites. Bar for A, B, and E–G, 20 μm. Bar for C, D–H, and I, 20 μm.

branched axonal processes (Fig. 3, E and F); this is not surprising, because we have previously demonstrated that KIF4 is not essentially required for process extension in hippocampal pyramidal neurons (3). On the other hand, the majority of the sh-GFP-KIF4 targeted neurons showed a dramatic reduction in P0 immunofluorescence signal along axons, with most of the labeling concentrated in the cell body (Fig. 3G and supplemental Fig. S2; see also supplemental Table S1). Such an effect was not observed after microinjection of control-sh-GFP (S-sh1 or S-sh3; Fig. 3, H–J, and supplemental Fig. S2). Interestingly, a decrease in Staufen staining was also observed along neurites of KIF4-suppressed neurons (Fig. 3, K and L).

KIF4 Mutations Alter the Intracellular Distribution of P0—To further test for the involvement of KIF4 in the anterograde trafficking of ribosomal constituents, we introduced two point mutations at the ATP-binding consensus motif in KIF4, similar to the one described for kinesin heavy chain and that result in a rigor kinesin with no motor activity (38). This KIF4 mutant remains localized to the cell body, along with ribosomal proteins P0 and S6 (supplemental Fig. S3), that were completely absent from neurites. The KIF4 tailless (Δ-CT) mutant was also used to analyze the distribution of ribosomal constituents. Ectopically expressed Δ-CT-GFP-KIF4 (Fig. 4A) localized to the cell body and bright fluorescence spots along axons, with a pattern indistinguishable from that of Myc-tagged KIF4-FL or endogenous KIF4. By contrast, in these neurons almost no staining for P0 (Fig. 4, B and C) or S6 (not shown) were detected along axons with most of the labeling found in the cell body. Quantitative measurements confirmed these observations (supplemental Table S1) and suggest that trafficking of ribosomal proteins, such as P0, to axons is dependent on the ATPase activity of the KIF4 motor domain and the cargo binding capacity of its tail domain. In addition, our results suggest that regions outside the tail domain are also responsible for the localization of KIF4 and P0 within axons; this may involve regions of KIF4 that serve as anchors. The possibility that dynein, kinesins, or myosins may switch from a dynamic motor to a static anchor has recently been suggested (39–41); some of these anchor activities may involve actin filaments or actin-associated proteins (41). Such a possibility was interesting to explore in the case of KIF4 for two reasons: 1) Immunofluorescence observations in CHO cells clearly established the presence of KIF4 in the actin-rich peripheral regions of the cell (this study) and 2) the fact that protein synthetic machinery and molecular motors have been detected associated with actin filaments at the cell periphery (41). Therefore, we search the KIF4 sequence to find out regions with the potential to serve as anchors. The results of this analysis revealed the presence within the second coiled-coiled domain of KIF4 of a region (amino acids 661–796) with homology to a domain common to members of the 4.1 protein superfamily termed the FERM (four point-one ERM) domain (supplemental Fig. S4). This domain, present at the N terminus of ERM, binds to membrane proteins, such as the cell adhesion molecule, L1 (42). Because previous studies have implicated KIF4 in the anterograde transport of L1 to axons (3), the possibility exists that
KIF4 may use this ERM-like domain to interact with L1-containing vesicles during transport and/or with L1 or other membrane proteins at the plasma membrane to serve as a static anchor. Therefore, in the final set of experiments and to test this idea, we generated a deletion mutant of KIF4 (Δ-ERM-KIF4) lacking amino acids 661–796 and analyzed the distribution of KIF4, L1, and P0 in DRGs. Transfection of DRGs with Δ-ERM-KIF4 fused to GFP at the N terminus revealed a uniform distribution of the molecule within axons with very few clusters of intense fluorescence (Fig. 4E). This pattern, which is completely different from that of endogenous KIF4 or Myc-KIF4-FL, or Δ-CT-KIF4, was associated with a similar change in the distribution of endogenous P0 (Fig. 4F and supplemental Table S1) or ectopically expressed HA-tagged P0 (not shown). By contrast, RNAi suppression of KIF4, or expression of the Δ-ERM-KIF4 mutant dramatically reduced L1 labeling from axons, with most of the staining localized to the cell body or initial axonal segments (Fig. 5, A–D); such an effect was not observed after expression of the S-sh3 RNAi plasmid (supplemental Fig. S5). The interaction of KIF4 with L1 was confirmed by immunoprecipitation of membrane fractions from CHO cells transfected with Myc-tagged KIF4-FL or Δ-CT KIF4 and L1-GFP, but not in cells coexpressing Myc-tagged Δ-ERM KIF4 and L1-GFP (Fig. 5E).

**DISCUSSION**

In neurons, the transport and targeting of RNA granules and ribosomes to dendrites or dendritic spines is an important sorting mechanism that has been implicated in local synaptic plasticity including spine development and long term changes in synaptic strength (43–45). In addition, during recent years a growing body of evidence has accumulated showing that translational machinery is also present in growing axons where it plays crucial roles in axon guidance, regeneration, survival, and presynaptic plasticity (20, 21, 24–27). It was reported that RNA dendritic transport requires microtubule-dependent motors, such as KIF5b (2, 28) but how motors interact with ribosomal constituents during trafficking to either dendrites or axons remains largely unknown. The present study provides new evidence about the function of KIF4 in developing neurons. In particular our results implicate KIF4 in the anterograde translocation and positioning of ribosomal constituents to developing axons. First, we showed by different approaches that the tail domain of KIF4 interacts with P0 and that its deletion or RNAi suppression dramatically reduced P0 immunolabeling from axons, with a concomitant accumulation at the cell body. Second, we showed that mutations of the ATP-binding site in KIF4, which should generate a rigor KIF4, produce a similar phenotype. Taken together, our observations suggest that by binding to P0 through its tail domain and using its motor activity, KIF4 is involved in the anterograde trafficking of P0 to axons. The phosphoprotein P0 is a structural and functional protein of the 60 S ribosome subunit that forms with the acidic proteins P1 and P2 a pentameric complex called the “stalk.” This structure is a lateral protuberance located in the active part of the ribosome particle, where interactions between mRNAs, tRNAs, and translation factors take place during the course of protein synthesis (46). Interestingly, the stalk links the GTPase-associated domain at the 28 S rRNA with cytoplasmic factors, such as EF1α, a candidate for binding mRNA to actin filaments at sites of mRNA anchoring (47). Therefore, the KIF-4 mediated translocation of P0 into developing axons could be of great importance because of the possibility of local translation at this subcellular compartment. It is likely that P0 is transported to axons as a macromolecular complex or perhaps as a component of an assembled...
Ribosome, because the trafficking of other ribosomal constituents (e.g., P1, P2, S6, and Staufen) display similar changes in distribution after KIF4 suppression or expression of KIF4 mutants. The association of KIF4 with P0 and its involvement in the trafficking of ribosomal constituents does not exclude the participation of other molecular motors. For example, periaxoplasmic ribosomal plaques are discrete ribosome-containing domains distributed intermittently along the periphery of axoplasm in myelinated fibers (48); immunofluorescence studies have revealed the presence of the microtubule-based motor, KIF3a, and myosin Va in these structures; however, whether they are directly involved in the transport of ribosomes or RNA granules remains to be established.

Our results also suggest another novel and unexpected function for KIF4 that involves an ERM-like domain located in its second coiled-coiled region. ERM domains interact with membrane proteins such as L1. Because KIF4 is involved in the anterograde translocation of L1 (Ref. 3 and this study), it may use its ERM-like domain to bind L1-containing vesicles during transport. In accordance with this, deletion of this domain results in accumulation of L1 at the cell body and reduction of labeling within axons. Interestingly, this domain appears to serve as an anchor for the clustering of KIF4 and ribosomal constituents within axons. Thus, whereas Δ-ERM-KIF4 enter the axons, its does not cluster along axons showing a diffuse distribution, which is also detected for P0 and other ribosomal constituents.

One likely interpretation of these observations is that KIF4 may not release P0 after reaching its final destination but interact with membrane proteins associated with the subcortical cytoskeleton and therefore serve as a static anchor for ribosomal constituents. Such a mechanism could explain the ribosomal clusters present in periaxoplasmic ribosomal plaques. Whether the clustering of KIF4 and ribosomal constituents involves an interaction of the KIF4 ERM-like domain with membrane L1 or with other membrane proteins remains to be established. In conclusion, our results provide new evidence suggesting that KIF4 is a multifunctional modular microtubule-based motor, with different domains involved in binding to distinct cargos (e.g. L1 and P0) and also capable of switching to a static anchor.

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