GRIF-1 and OIP106, Members of a Novel Gene Family of Coiled-Coil Domain Proteins

ASSOCIATION IN VIVO AND IN VITRO WITH KINESIN*

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γ-Aminobutyric acid agonist receptor-interacting factor (GRIF-1) is a 913-amino acid protein proposed to function as a GABA-A receptor β-subunit-interacting, trafficking protein. GRIF-1 shares ~44% amino acid sequence identity with O-linked N-acetylgalcosamine transferase aromatic protein 106, OIP106. Both proteins contain predicted coiled-coil domains and probably constitute a novel gene family. The Drosophila orthologue of this family of proteins may be Milton. Milton shares ~44% amino acid homology with GRIF-1. Milton is proposed to function in kinesin-mediated transport of mitochondria to nerve terminals. We report here that GRIF-1 and OIP106 also associate with kinesin and mitochondria. Following expression in human embryonic kidney 293 cells, both GRIF-1 and OIP106 were shown by co-immunoprecipitation to be specifically associated with an endogenous kinesin heavy chain species of 115 kDa and exogenous KIF5C. Association of GRIF-1 with kinesin was also evident in native brain and heart tissue. In the brain, anti-GRIF-1 (1–633) antibodies specifically co-immunoprecipitated two kinesin-immunoreactive species with molecular masses of 118 and 115 kDa, and in the heart, one kinesin-immunoreactive species, 115 kDa, was immunoprecipitated. Further studies revealed that GRIF-1 was predominantly associated with KIF5A in the brain and with KIF5B in both the heart and in HEK 293 cells. Yeast two-hybrid interaction assays and immunoprecipitations showed that GRIF-1 associated directly with KIF5C with the GRIF-1/KIF5C interaction domain localized to GRIF-1-(124–283). These results further support a role for GRIF-1 and OIP106 in protein and/or organelle transport in excitable cells in a manner analogous to glutamate receptor-interacting protein 1, in the motor-dependent transport of α-amino-3-hydroxy-5-methylisoxazole-4-propionate glutamate excitatory neurotransmitter receptors to dendrites.

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γ-Aminobutyric acid agonist receptor-interacting factor (GRIF-1) is a 913-amino acid protein first identified as a protein expressed in rat brain that has been proposed to function as a GABA-A receptor-trafficking factor (1). GRIF-1 was also recently identified as a protein that interacts with the enzyme uridine diphosphate-N-acetylgalcosamine:polypeptide β-N-acetylgalcosaminyltransferase or O-GlcNac transferase (OGT) (2, 3). OGT catalyzes the post-translational modification of proteins by β-O-linked N-acetylgalcosamine (GlcNAc) in the cell cytoplasm. GRIF-1 thus has the alternative name OGT-interacting protein 98 (OIP98). GRIF-1 (OIP98) shares ~44% amino acid sequence identity over the full-length sequence with the human protein, OIP106, also known as KIAA1042 (4). OIP106 was also shown to associate with OGT, suggesting that GRIF-1 (OIP98) and OIP106 form a new gene family (1, 2).

The human orthologue of GRIF-1 (OIP98) is the protein encoded by the gene ALS2CR3 (5), a protein of unknown function. The Drosophila orthologue is probably Milton (6). Milton shares ~44% amino acid homology with GRIF-1 (OIP98). Milton, like GRIF-1, is enriched in neuronal tissue. It was identified originally by a genetic screen of Drosophila mutations, searching for effects on the cell biology of the axon and synaptic terminals. Milton is a mitochondria-associated protein (6, 7). The microtubule-based motor protein, kinesin, was found to be co-immunoprecipitated with Milton from extracts of fly heads, thus suggesting that it is required for the kinesin-mediated transport of mitochondria to nerve terminals.

Here we report that GRIF-1 (OIP98) and OIP106 also co-associate with kinesin and mitochondria, thus demonstrating that Milton, GRIF-1, and OIP106 are indeed species orthologues, further supporting a role for GRIF-1 in protein and/or organelle transport in excitable cells.

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EXPERIMENTAL PROCEDURES

Constructs and Antibodies—pCI5GRIF-1, pGAD7GRIF-1-(1–913), pGAD10GRIF-1-(8–633), and pGAD7GRIF-1-(124–283), pMBl-33GABA receptor β-subunit intracellular loop (residues 303–427), and affinity-purified anti-GRIF-1 (1–833) antibodies were as previously described (1). Full-length cDNA clones of KIAA1042-(1–953) (hereafter termed OIP106-(1–953)) and KIAA0531 (kinesin heavy chain, KIF5C-(1–957); hereafter termed KIF5C-(1–957)), cloned into the Sall/Not restriction sites of the pBluescript II SK+ vector, were obtained from Professor T. Nagase (Kazusa Research Institute, Chiba, Japan). Each insert was excised by restriction enzyme digestion and used as template DNA for PCR amplification and cloning into pCR1 TOPO (Invitrogen, Groningen, The Netherlands). Specific oligonucleotide primers incorporating the restriction sites for EcoRI and BamHI were used to amplify OIP106, and primers containing the restriction sites EcoRI and Sall were used to amplify KIF5C. The full-length OIP106...
and KIF5C clones were subcloned from the respective pCRII TOPO constructs in frame into the modified (m) LexA vectors pGADT7 and pMBL33 to generate the fusion constructs pGADT7OIP106-(1–953) and pMBL33KIF5C. KIF5C was also subcloned into the mammalian expression vector, pCIS, to generate pCISGRIF-1 and into pCMVTag4a engineered to include an N-terminal c-Myc tag; KIF5C was subcloned into pCDNAHisMax (Invitrogen) to generate pCDNAHisMaxKIF5C. GRIF-1-(283–913) and OIP106-(124–283) were obtained by PCR amplification using pGADT7GRIF-1 and pGADT7OIP106 as templates, respectively, and cloned into the EcoRI/XhoI restriction sites of the pGADT7 vector (Clontech, Palo Alto, CA) to generate pGADT7GRIF-1-(283–913) and pGADT7OIP106-(124–283). GRIF-1-(283–913), GRIF-1-(124–283), and OIP106-(124–283) were obtained by PCR amplification using pGADT7GRIF-1 and pGADT7OIP106 as templates, respectively, and cloned into the EcoRI/XhoI restriction sites of a modified pCMV4a plasmid to generate pCMVGRIF-1-(283–913), pCMVGRIF-1 (124–283), and pCMVTag4a (124–283), where each protein was N-terminally c-Myc tagged. All constructs were verified by DNA sequencing using the ABI PRISM 310 Genetic Analyser. Further, for all constructs used in yeast two-hybrid interaction assays, the expression of fusion proteins was verified by immunoblotting (data not shown).

The peptide CNSGOSLSLQGLRRmQs that corresponds to GRIF-1-(574–587) (N-terminal cysteine for chemical modification) via the cysteine residue, and both rabbit and sheep anti-GRIF-1-(574–889) polyclonal antibodies were generated as described (8). Anti-kinasin monoclonal antibodies were from Cytokeleton Inc. (Denver, CO); anti-β-actin and anti-β-tubulin monoclonal antibodies were from Chemicon International Inc. (Temecula, CA); anti-His G antibodies were from Invitrogen; and anti-His G antibodies were from Abcam Ltd. (Cambridge, UK). Affinity-purified anti-c-Myc antibodies were from Upstate (Charlottesville, VA); and anti-dynein monoclonal antibodies were from Chemicon International Inc. (Temecula, CA).

The plasmid pMBL33 to generate the fusion constructs pGADT7OIP106-(1–953) and pGADT7GRIF-1-(124–283), where each protein was N-terminally c-Myc tagged. All constructs were verified by DNA sequencing using the ABI PRISM 310 Genetic Analyser. Further, for all constructs used in yeast two-hybrid interaction assays, the expression of fusion proteins was verified by immunoblotting (data not shown).

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RESULTS

Anti-GRIF-1 Antibody Specificities—GRIF-1 and OIP106 have 42% amino acid identity overall but 52% identity over the sequence GRIF-1-(8–633) that was used for the production of anti-GRIF-1 antibodies. It is thus probable, as suggested by Iyer et al. (2), that anti-GRIF-1 antibodies recognize both GRIF-1 and OIP106. Indeed, when each was expressed alone in HEK 293 cells, anti-GRIF-1-(8–633) antibodies recognized three molecular mass species of 115, 106, and 98 kDa as reported previously for GRIF-1-transfected cells (1) and two molecular mass species of 170 and 115 kDa for OIP106-transfected cells (Fig. 1). OIP106 is a polypeptide consisting of 953 amino acids; therefore, the 115-kDa species is the monomeric form of 115 kDa. Because of the observed molecular mass species of 115, 106, and 98 kDa for OIP106, it can clearly be seen that anti-GRIF-1-(8–633) antibodies recognize GRIF-1 only and that anti-kinesin antibodies recognize KIF5C but not GRIF-1. The positions of molecular weight standards (kDa) are shown on the right.

GRIF-1 and OIP106 Both Co-associate with Endogenous Kinesin in Heterologous Expression Systems and in the Brain—Affinity-purified anti-GRIF-1-(8–633) antibodies successfully immunoprecipitated both GRIF-1 and OIP106 from detergent extracts of HEK 293 cells transfected separately with each of the individual respective clones (Fig. 2). Since Milton was found to co-immunoprecipitate with kinesin, it was of interest to determine whether such an association occurred in mammalian systems. In Fig. 2, it can be seen that indeed, using a broad spectrum anti-kinesin heavy chain antibody, kinesin immunoreactivity, 115 kDa, was found in immune pellets resulting from precipitation with anti-GRIF-1-(8–633) antibodies for both GRIF-1- and OIP106-transfected cells. No anti-kinesin immunoreactivity was detected in control immunoprecipitations where the primary antibody was nonimmune Ig (Fig. 2). Further specificity control experiments using yeast lysates expressing either GRIF-1 or KIF5C showed that anti-kinesin antibodies do not recognize GRIF-1 and vice versa (i.e., anti-GRIF-1 antibodies do not recognize kinesin) (Fig. 1).

GRIF-1 and kinesin were also found to co-associate in native brain tissue. The results are shown in Fig. 3. Anti-GRIF-1-(8–633) antibodies immunoprecipitated the three molecular weight species observed in the immunoblots. Probing with anti-GRIF-1-(874–889) confirmed that the M_r 115,000 immunoreactive band of 115 kDa was indeed GRIF-1, and probing with anti-kinesin antibodies revealed the presence of kinesin in anti-GRIF-1-(8–633) but not control immune pellets. In the brain, two kinesin-immunoreactive bands of 118 and 115 kDa were detected (Fig. 3). An additional control using an anti-Kv1.2 potassium channel antibody showed no immunoreactivity in test and control immune pellets. Further, immune pellets from both the GRIF-1 HEK 293-transfected cells and the brain were also screened for reactivity with anti-β-actin, anti-β-tubulin, and anti-dynein antibodies. All antibodies yielded robust signals in detergent-solubilized extracts; however, no immunoreactivity was detected in the immune pellets for any of these three antibodies (results not shown).
The Association between GRIF-1 and Kinesin is Direct: Demonstration and Specificity Using the Yeast Two-hybrid Interaction Assay—Association of two proteins by co-immunoprecipitation does not demonstrate definitively that two proteins interact directly, since they may co-associate via an intermediary adaptor protein molecule. Thus, to test whether GRIF-1 and OIP106 do associate directly with kinesin, yeast two-hybrid interaction assays were carried out.

Kinesin is a member of the kinesin superfamily of proteins (i.e. the KIFs). In the human genome, there are 45 KIF members; 38 of these are expressed in the brain (11). The anti-kinesin antibody employed initially in the immunoprecipitation studies was a monoclonal antibody, SUK4, which was raised against the 130-kDa kinesin heavy chain of sea urchin egg (12). This antibody was originally reported to cross-react with a 120-kDa heavy chain of bovine brain kinesin; hence its use in mammalian species (12). However, more detailed information about the KIF subtype specificity of SUK4 is unfortunately unavailable. In the absence of this knowledge, KIF5C was selected for the yeast two-hybrid studies, since it is described as neuron-specific (11). Also, the predicted size of KIF5C is similar to that of the kinesin-immunoreactive bands seen in the co-immunoprecipitation experiments (Figs. 2 and 3). The results of the yeast two-hybrid interaction assays are summarized in Table I.

Co-transformation of yeast with pGADT7GRIF-1-(1–913) with pMBL33KIF5C-(1–957) revealed a weak but significant interaction of the resulting two fusion proteins as determined by both nutritional selection on SD/H11002W, H11002L, H11002H media and H9252-galactosidase reporter gene activity. The GRIF-1 truncated constructs, GRIF-1-(8–633) and GRIF-1-(124–283), were also found to interact with KIF5C, whereas GRIF-1-(283–913) did not interact. This therefore identifies the kinesin binding domain of GRIF-1 to within residues 124–283. This region coin-
The results are representative of those described under "Experimental Procedures." Colony growth is shown as follows. (+), <10 cfu; (+), 10–100 cfu; ++, 100–200 cfu; +++, >200 cfu. The results are representative of n = 3 independent co-transformations.

| AD vector | DNA BD vector | Growth on –W, –L dropout medium | Growth on –W, –L –H dropout medium | β-Galactosidase activity |
|-----------|--------------|---------------------------------|-----------------------------------|--------------------------|
| pGADT7GRIF-1-(1–913) | pMBL3KIF5C-(1–957) | ++     | (+)     | (+)     |
| pGAD10GRIF-1-(8–633) | pMBL3 KIF5C-(1–957) | ++     | ++      | +       |
| pGADT7GRIF-1-(283–913) | pMBL3 KIF5C-(1–957) | ++     | –       | –       |
| pGADT7GRIF-1-(124–283) | pMBL3 KIF5C-(1–957) | ++     | ++      | ++      |
| pGADT7OIP106-(124–283) | pMBL3 KIF5C-(1–957) | ++     | –       | –       |
| pGADT7GRIF-1-(1–913) | pMBL33 | ++     | –       | –       |
| pGAD10GRIF-1-(8–633) | pMBL33 | ++     | –       | –       |
| pGADT7GRIF-1-(124–283) | pMBL33 | ++     | –       | –       |
| pGADT7OIP106-(1–953) | pMBL33 | ++     | –       | –       |
| pGADT7OIP106-(124–283) | pMBL33 | ++     | –       | –       |
| pGAD7T | pMBL33 | ++     | –       | –       |
| pGAD7T | pMBL33 | ++     | –       | –       |
| pGAD10GRIF-1-(8–633) | pMBL33 β2-IL | ++     | ++      | +       |

Table I

GRIF-1 and the KIF protein, KIF5C, interact directly: Demonstration by yeast two-hybrid interaction assays

The yeast strain L40 was co-transformed with bait and fish constructs and transformants grown on –2 SD (i.e., –W, –L, and –H). Colonies from –2 plates were restreaked onto new plates, and β-galactosidase activity was measured by filter lift assays all as described under "Experimental Procedures." Colony growth is shown as follows. (+), <10 cfu; (+), 10–100 cfu; ++, 100–200 cfu; +++, >200 cfu. The results are representative of n = 3 independent co-transformations. Further Investigation into the Specificity of Interaction between Native and Recombinant GRIF-1 and the KIF5 Proteins—Since both the yeast two-hybrid results and the co-expression of GRIF-1 and N-terminal His-tagged KIF5C in HEK 293 cells suggested a direct association between GRIF-1 and KIF5C, further experiments were carried out to determine the KIF interaction specificity of native GRIF-1. Thus, Triton X-100 extracts of homogenates of rat forebrain or HEK 293 cells transfected with pCISGRIF-1 or pCISOIP106 were prepared and immunoprecipitated with anti-GRIF-1-(8–633) antibodies, and the immune pellets were analyzed by immunoblotting using anti-KIF5A, KIF5B, and KIF5C antibodies. The results are shown in Figs. 5 and 6. As before, both GRIF-1-(8–633) and GRIF-1-(874–889) immunoreactivity was detected in the immune pellets. In the brain, KIF5A and KIF5C were the most abundantly expressed, but all three KIF antibodies recognized the appropriate molecular mass immunoreactive species in Triton X-100 extracts (i.e., KIF5A (118 kDa), KIF5B (115 kDa), and KIF5C (115 kDa)). A strong immunoreactive signal for KIF5A was detected in the immune pellets, demonstrating an association between GRIF-1 and KIF5A. Weak signals of similar intensity were detected for both KIF5B and KIF5C (Fig. 5).

In HEK 293 cells, only KIF5B was detected in the detergent extracts, but it was specifically immunoprecipitated from both GRIF-1- and OIP106-transfected cell homogenates (Fig. 6). A weak band was seen with ~115 kDa in the immune pellet of GRIF-1-transfected cells probed with anti-KIF5C antibodies that could be KIF5C. A lack of detection in the detergent extract may be due to sensitivity; the primary antibodies are incubated with ~30 times the amount of protein applied to the immunoblot for the detection of immunoreactivity in the detergent extracts. Other bands seen in the immunoblot are probably IgG molecules.

Association of Anti-GRIF-1-(8–633) Immunoreactivity with Endogenous Kinesin in the Heart—We previously reported that anti-GRIF-1-(8–633) immunoreactivity was found only in excitable tissues (1). Notably, as well as the 115- and 106-kDa GRIF-1-immunoreactive species, an 88-kDa immunoreactive band was expressed in the heart. It was suggested that this may be the GRIF-1b C-terminal truncated splice variant; GRIF-1a is the nomenclature for the 913-amino acid protein (1). It was thus of interest to investigate the possible association of GRIF-1 with kinesin in the heart. In Fig. 7, it is shown that anti-GRIF-1-(8–633) antibodies immunoprecipitated the three major bands of 115, 106, and 87 kDa observed in original immunoblots from detergent extracts of the heart (1). When these immune pellets were probed with anti-kinesin antibodies, a single immunoreactive band of 115 kDa was detected compared with the two immunoreactive bands, 118 and 115 kDa, observed for brain. Liver was used as a negative control.
since it was previously reported that no anti-GRIF-1 immuno-reactivity was detected in soluble, P2, or nuclear crude subcellular fractions (1). Here, detergent extracts of whole cell homogenates were used for the immunoprecipitations, and weak anti-GRIF-1-(8–633) immunoreactivity was found in both homogenates and immune pellets. Kinesin immunoreactivity was not detectable in the pellets.

The kinesin specificity was investigated by probing the anti-GRIF-1-(8–633) immune pellets with the anti-KIF5A, -5B, and -5C antibodies. The results are shown in Fig. 7, C–G. First, anti-GRIF-1-(874–889) recognized two bands of 118 and 115 kDa in the immune pellets, showing that GRIF-1 was present. The 88-kDa species was not detected by the GRIF-1 C-terminal antibodies, but this was expected, since the GRIF-1b C-termi-
nal truncated splice form comprises amino acids 1–672 and therefore does not contain the GRIF-1-(874–889) peptide epitope. KIF5B and KIF5C were both expressed in heart tissue, but only KIF5B (115 kDa) immunoreactivity was detected in the immune pellets (Fig. 7). Thus, the band seen with the pan kinesin antibody is probably KIF5B.

Do GRIF-1 and OIP106 Associate with Mitochondria following Expression in HEK 293 Cells?—The Drosophila protein, Milton, was proposed to be a mitochondria-associated protein required for kinesin-mediated transport of mitochondria to nerve terminals (6). We have shown here that GRIF-1 and its homologue, OIP106, both associate with kinesin, thus establishing that Milton is indeed a species ortholog of GRIF-1 and OIP106. It was thus of interest to determine whether GRIF-1 and OIP106 also associated with mitochondria. HEK 293 cells were transfected with either GRIF-1, OIP106, or Milton clones. The distribution of mitochondria and the expressed proteins was determined post-transfection using MitoTracker Red CMXRos and the appropriate respective antibodies. The results are shown in Fig. 8. For Milton, similar results to those reported by Stowers et al. (6) were found (i.e. Milton-transfected cells showed a tight aggregation of mitochondria that co-local-
Co-association of GRIF-1 and OIP106 with Kinesin

FIG. 8. Localization of GRIF-1, OIP106, Milton, and mitochondria in HEK 293 cells transfected with pCISGRIF-1, pCISOIP106, and pCMVtag1milton. HEK 293 cells adhered to poly-l-lysine-coated coverslips were transfected with the appropriate clones by the calcium phosphate method, incubated for 20 h, and stained with MitoTracker Red CMXRos and anti-GRIF-1-(8–633) or anti-OGT. Milton 5A 124 antibodies, all as described under “Experimental Procedures.” A–L, a single confocal section; A–D, pCMVtag1milton-transfected cells; E–H, pCISGRIF-1-transfected cells; I–L, pCISOIP106-transfected cells. A, E, and I, staining with MitoTracker Red CMXRos; B, staining with anti-O-GlcNAc-glycosylated proteins by OGT. Milton 5A 124 antibodies; F and J, staining with anti-GRIF-1-(8–633) antibodies; C, A and B merged; G, E and F merged; K, I, and J merged; D, H, and L, enlargements of the boxes depicted in C, G, and K, respectively, where yellow shows areas of co-localization. In the field shown in I, note the clear difference in distribution of mitochondria found for both transfected (green cells) and nontransfected cells. The results shown are representative of multiple observations found for n = 2 independent transfections. Scale bars, 5 µm.

DISCUSSION

GRIF-1 and OIP106 are thought to be members of a novel coiled-coil domain family by virtue of the similarity in their primary structures (1, 2). Their function is unknown. It was speculated that GRIF-1 is a neuronal trafficking molecule because of its enrichment in excitable tissues, its association with β, subunit-containing GABA, receptors, and its homology with other proteins such as huntingtin-associated protein (HAP-1). HAP-1 binds to the p150flied subunit of dynactin, glutamate neurotransmitter receptor-GluR2-interacting protein (GRIP1), the inositol 1,4,5-trisphosphate receptor type 1, and kinesin 5C (see Refs. 13–17, respectively). It was reported to play a role in the regulation of vesicular trafficking from early endosomes to late endocytic compartments (18). More recently, HAP1 was shown to be involved with huntingtin in the enhancement of vesicular transport of brain-derived neurotrophic factor along microtubules (17). Both GRIF-1 and OIP106 also associate with the enzyme OGT (2). Many classes of proteins are post-translationally, dynamically modified by O-glycosylation catalyzed by OGT. O-GlcNAc-glycosylated proteins are particularly abundant in the central nervous system (e.g. see Ref. 19). These proteins include transcription factors. Indeed, Iyer et al. (2) showed that OIP106 formed an in vivo RNA polymerase II-OIP106-OGT complex, leading to the speculation that OIP106 may target OGT to transcriptional complexes. Interestingly, HAP-1 and huntingtin have also been shown to associate with the transcription factor, NeuroD (20), thus revealing further similarities between HAP-1 and the GRIF-1/OIP106 coiled-coil domain family of proteins.

In this paper, we have identified a second family of proteins that associate with both GRIF-1 and OIP106. We have shown by co-immunoprecipitation strategies that GRIF-1 and its homologue, OIP106, both interact in vitro and in vivo (GRIF-1) with kinesin. Furthermore, OIP106 and possibly GRIF-1 aggregate mitochondria following their expression in mammalian cells. These results provide further support of a role for both GRIF-1 and OIP106 in membrane trafficking of vesicles and/or organelles utilizing molecular motor proteins. Additionally, they provide evidence that the Drosophila protein, Milton, is indeed a species orthologue of GRIF-1 and OIP106.

The impetus for the experiments described herein came from the work of Stowers et al. (6), who showed that kinesin heavy chain was associated with Milton following immunoprecipitation from extracts of Drosophila heads by two different specificity anti-Milton antibodies. Kinesins belong to the kinesin superfamily (i.e. KIFs). KIFs are microtubule-based mechanochemical motors that are involved primarily in the anterograde transport of organelles and protein complexes. The kinesin molecule is a heterotrimer consisting of two kinesin heavy chains and two light chains; each KIF has its own cargo. The kinesin heavy chain has three functional domains, the motor domain, an α-helical stalk domain, and a globular tail region. The motor domain has two conserved sequences proximal to a Walker A ATP binding motif and a microtubule binding domain (11). Several KIFs attach to specific cargoes through interactions with adaptor proteins in nonmotor regions. Stowers et al. (6) suggested that Milton functions as an adaptor protein in the transport of mitochondria to synapses. However, they were
unable to demonstrate a direct association between Milton and kinesin, which led them to suggest that an additional linker protein may be required to form an adaptor protein complex (6). It was stated that a precedent for such a complex was the finding that KIF17 forms a complex with mLin10 in the transportation of N-methyl-D-aspartate receptor NR2B subunits to the synapse (21, 22). In contrast to Ref. 6, we were able to demonstrate a direct association between GRIF-1 and KIF5C in a yeast two-hybrid interaction assay (Table I). However, association between full-length GRIF-1 and KIF5C was weak compared with strong reporter gene activation observed for the GRIF-1 fragment, GRIF-1-(124–283), that contains a coiled-coil domain. Interestingly, despite robust expression, the corresponding OIP106 coiled-coil domain, OIP-(124–283), that shares 60% amino acid identity with GRIF-1-(124–283) did not result in reporter gene activity following co-transformation with KIF5C. It was found, however, that OIP106, like GRIF-1, did immunoprecipitate with exogenous KIF5C following over-expression of both in HEK 293 cells. It is difficult to rationalize these two observations. In a yeast two-hybrid interaction assay, bait and fish proteins may be concluded to associate, but this may be due to an intermediary protein supplied by the yeast cells. Thus, either the interaction between GRIF-1 or OIP106 and kinesin is indirect and, in yeast, GRIF-1 but not OIP106 binds directly to KIF5C. In this case, OIP106 must bind to an intermediary endogenous protein in HEK 293 cells. Given the high degree of amino acid identity between the KIF5C binding domain of GRIF-1 and the homologous region of OIP106 (80% similarity), one would predict it to be unlikely that the proteins would behave differently. It should be noted, however, that OIP106 does not associate with the intracellular loop of the GABAA receptor β2 subunit in yeast two-hybrid assays (23). Thus, whereas GRIF-1 and OIP106 both associate with OGT and kinesin, there may be subtle differences with regard to interactions with other proteins that may reflect dissimilarities in their respective functions.

GRIF-1 and/or OIP106 are promiscuous with regard to association with KIF5 subtypes. In whole brain tissue, GRIF-1 is associated predominantly with KIF5A, although KIF5C is abundantly expressed; in the heart, association is with KIF5B, although both KIF5B and KIF5C are highly expressed; in HEK 293 cells, association of both proteins is with endogenous KIF5B, although both were found to associate with exogenous KIF5C. Kanai et al. (24) suggested that there is functional redundancy between KIF5A, KIF5B, and KIF5C. The three proteins share at least 63 and 73% amino acid sequence identity and similarity, respectively; thus, it is probable that each may contain a GRIF-1 binding domain within a conserved region. In general, kinesin heavy chains are thought to bind cargo in their C-terminal nonmotor regions. A ClustalW alignment of the primary structures of KIF5A, -5B, and -5C shows that within the nonmotor region, they do indeed share at least 59 and 71% amino acid identity and similarity, respectively. Preferential association with KIF5A in the brain may be due to the co-localization within defined neuronal cell types of GRIF-1 and KIF5A. Since the studies described herein use detergent extracts of whole brain homogenates for immunoprecipitations, they would not distinguish between different neuronal cell populations. In the heart, preferential association with KIF5B may be explained by a higher affinity of KIF5B for GRIF-1.

The protein GRIP1 is an α-amino-3-hydroxy-5-methylisoxazole-4-propionate glutamate neurotransmitter receptor-GluR2-interacting protein. GRIP1 was shown to interact directly with kinesin heavy chains, and thus this complex provided the molecular motor and steer to traffic α-amino-3-hydroxy-5-methylisoxazole-4-propionate receptors to dendrites in neurons (25). Based on the findings described in this paper and by Beck et al. (1) together with those of the groups of Schwarz (6) and Hart (2, 3) we propose that Milton, GRIF-1, and OIP106 may all share a similar function to GRIP-1 and...
possibly HAP-1 (i.e., the anterograde trafficking of organelles such as mitochondria or vesicles containing receptor molecules to synaptic terminals). Interestingly, recently Li et al. (26) demonstrated that there is a high level of dynamism in dendritic mitochondrial distribution associated with synaptic activity and synaptic morphogenesis. It may be speculated that GRIF-1 and OIP106 play a pivotal role in the trafficking of mitochondria in concert with receptor-containing vesicles, ensuring fidelity of synaptic function during development and synaptic activity. A schematic model for the action of GRIF-1 and OIP106 is shown in Fig. 9.

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