Rab11 small G protein has been implicated in vesicle recycling, but its upstream regulators or downstream targets have not yet been identified. We isolated here a downstream target of Rab11, named rabphilin-11, from bovine brain. Moreover, we isolated from a rat brain cDNA library its cDNA, which encoded a protein with a Mr of 100,946 and 908 amino acids (aa). Rabphilin-11 bound GTP-Rab11 more preferentially than GDP-Rab11 at the N-terminal region and was specific for Rab11 and inactive for other Rab and Rho small G proteins. Both GTP-Rab11 and rabphilin-11 were colocalized at perinuclear regions, presumably the Golgi complex and recycling endosomes, in Madin-Darby canine kidney cells. In HeLa cells cultured on fibronectin, both the proteins were localized not only at perinuclear regions but also along microtubules, which were oriented toward membrane lamellipodia. Treatment of HeLa cells with nocodazole caused disruption of microtubules and dispersion of GTP-Rab11 and rabphilin-11. Overexpression of the C-terminal fragment of rabphilin-11 (aa 607–730), lacking the GTP-Rab11 binding domain, in HeLa cells reduced accumulation of transferrin at perinuclear regions and cell migration. Rabphilin-11 turned out to be a rat counterpart of recently reported bovine Rab11BP. These results indicate that rabphilin-11 is a downstream target of Rab11 which is involved in vesicle recycling.

The Rab family small G proteins, consisting of more than 30 members, regulate intracellular vesicle trafficking, including exocytosis, endocytosis, and recycling (for reviews, see Refs. 1–4). We purified a small G protein with a Mr of about 24,000 from the microsome-Golgi fraction of rat liver in 1990 (5) and cloned its cDNA in 1991 (6). The sequence analysis revealed that this small G protein is a rat counterpart of canine Rab11, which had been cloned from a MDCK1 cell cDNA library by Chavrier et al. (7) in 1990. Rab11 is ubiquitously expressed but particularly enriched in tissues with active vesicle trafficking (6, 7). Rab11 has subsequently been shown to be localized at sorting endosomes, recycling endosomes, the trans-Golgi network membranes, and the post-Golgi secretory vesicles, and to be implicated in vesicle recycling (8–11). However, the mode of action of Rab11 in vesicle recycling has not yet been clarified.

The Rab family small G proteins have two interconvertible forms, the GDP-bound inactive and GTP-bound active forms (3, 4). The conversion of the GDP-bound form to the GTP-bound form is regulated by two types of upstream regulators, GEPs and GDIs. The reverse conversion is regulated by GTPase-activating proteins. Each Rab family member appears to have its specific GEP and GTPase-activating protein, whereas GDIs bind to all the Rab family members. The GTP-bound form binds to downstream target(s) that exerts its function. Of the many Rab family members, the downstream targets of Rab3, -5, -6, -8, and -9, named rabphilin-3 (12, 13) and rim (14), rabaptin-5 (15) and EEA1 (16, 17), rabkinesin-6 (18), rab8ip (19), and p40 (20), respectively, have thus far been identified and characterized. However, neither the upstream regulators nor the downstream targets of Rab11 have been identified.

We isolated and characterized here a downstream target of Rab11. We named this protein rabphilin-11, because we previously isolated and characterized a downstream target of Rab3 and named it rabphilin-3 (philin for the Greek word for friend) (13).

**EXPERIMENTAL PROCEDURES**

[35S]GTPγS-GST-Rab11 Blot Overlay—GST-Rab11, -Rab3A, -Rab5, and -RhoA were purified from overexpressing *Escherichia coli* as described (12). [35S]GTPγS-GST-Rab11 blot overlay was done as described (21). Briefly, purified GST-Rab11 (40 pmol) was labeled with 1.25 μM [35S]GTPγS (1.4 × 104 cpm/pmol) by incubation at 30 °C for 20 min in a solution (40 μl) containing 18 mM Tris/His/Cl at pH 7.5, 8.1 mM EDTA, 0.9 mM DTT, 4.5 mM MgCl2, and 0.3% CHAPS. After this incubation, 4 μl of 100 mM MgCl2 was added to give a final concentration of 13 mM, and the mixture was immediately cooled on ice. Where indicated, [35S]GTPγS (1.4 × 104 cpm/pmol) was used instead of [35S]GTPγS. The sample to be tested was subjected to SDS-PAGE and transferred to a nitrocellulose membrane. The membrane was blocked at 4 °C in PBS containing 5 mM DTT, 50 mM ZnCl2, 0.1% Triton X-100, and 1 mg/ml BSA. The membrane was then incubated at room temperature for 5 min, followed by incubation at 4 °C for 10 min with 5 μl [35S]GTPγS-GST-Rab11 (70.0 × 104 cpm) in a solution (1 ml) containing 25 mM MES/NaOH at pH 6.5, 2.5 mM DTT, 50 mM NaCl, 1.25 mM MgCl2, and 1.25 mg/ml BSA. Where indicated, non-radioactive GTPγS-GST-Rab3A, -Rab5, or -RhoA (650 pmol each) was added to this solution. After the incubation, the membrane was washed with 25 mM MES/NaOH at pH 6.5 containing 50 mM NaCl, 5 mM MgCl2, and 0.05% Triton X-100, followed by autoradiography by use of an image analyzer (Fuji BAS-2000; Fuji Photo Film Co., Tokyo, Japan).

Rab11S25N, a Myc-tagged dominant negative mutant of Rab11; MBP, maltose-binding protein-tagged; GFP, green fluorescent protein; MES, 4-morpholinolinesulfonylic acid; CHAPS, 3-[3-cholamidopropyl]-dimethylammonio]-1-propanesulfonate.
Purification and Molecular Cloning of Rabphilin-11—All the following procedures were done at 4 °C. Bovine brains (250 g wet weight) were homogenized with a solution (300 ml) containing 20 mM Tris/HCl at pH 7.5, 2 mM EDTA, 0.32 M sucrose, and a protease inhibitor mixture (10 μM aprotinin, 1 mM phenylmethylsulfonyl fluoride, 20 μg/ml leupeptin, and 1 μg/ml pepstatin A). The homogenate (40 ml) was centrifuged for 1 h. The supernatant was stored at −80 °C until use. One-eighth of the supernatant (40 ml, 320 mg of protein) was applied on a Mono Q HR 10/10 column (Amersham Pharmacia Biotech) equilibrated with Buffer A (20 mM Tris/HCl at pH 7.5, 1 mM EDTA, 1 mM DTT, and 5 mM MgCl2). Elution was performed with a 240-ml linear gradient of NaCl (0–1M) in Buffer B (20 m M potassium phosphate at pH 7.0, 100 mM NaCl). Fractions of 4 ml each were collected and applied on a TSK gel phenyl-5PW RP column (0.75 cm × 30 cm; Calbiochem) equilibrated with Buffer C containing 1% cholate. Elution was performed with a 240-ml linear gradient of potassium phosphate (20–500 mM) in Buffer B. Fractions of 0.5 ml each were collected. Rabphilin-11 appeared in fractions 12–20. The active fractions (4.5 ml, 2 mg of protein) were collected and subjected to SDS-PAGE (8% polyacrylamide gel) and subjected to SDS-PAGE (8% polyacrylamide gel). The protein band corresponding to a protein with a Mr of about 140,000 was cut out from the gel and digested with a lysyl endopeptidase, and the digested peptides were separated by TSKgel PODS-80Ts (4.6 × 150 mm; Tosoh, Tokyo, Japan) equilibrated with 0.08% trifluoroacetic acid. Elution was performed with a 60-ml linear gradient of acetonitrile (0–80%) in 0.08% trifluoroacetic acid. Fractions of 0.5 ml each were collected. Rabphilin-11 appeared in fractions 78–83 (see Fig. 1). The active fractions (3 ml, 0.3 mg of protein) were collected and lyophilized. This lyophilized sample (0.3 mg of protein) was dissolved in 0.3 ml of Laemmli’s buffer and subjected to SDS-PAGE (8% polyacrylamide gel). The protein band coincided with a protein with a Mr of about 140,000 which was identified by protein staining (Fig. 1, lane 4). Antibodies and Immunofluorescence Staining—A rabbit polyclonal antibody against rabphilin-11 was raised against GST-rabphilin-11–3 (aa 53–301). The antisera was affinity purified with MBP-rabphilin-11–3 (aa 53–301) covalently coupled to CNBr-activated Sepharose (Amersham Pharmacia Biotech). Hybridoma cells expressing the mouse monoclonal anti-Myc antibody (9E10) were purchased from American Type Culture Collection (Rockville, MD). An anti-tubulin β antibody was purchased from Sigma. Second antibodies for immunofluorescence microscopy were obtained from Chemicon International, Inc. (Temecula, CA). Immunofluorescence microscopy was performed as described (23). Briefly, the cells used here were fixed in 3.7% formaldehyde/PBS for 20 min. The fixed cells were permeabilized with 0.2% Triton X-100/PBS for 10 min. After being soaked in 10% FCS/PBS for 1 h, the cells were treated with the first antibody in 10% FCS/PBS for 1 h. The cells were then washed with PBS three times, followed by incubation with the second antibody in 10% FCS/PBS for 1 h. The cell preparations were washed with PBS three times, the cells were examined by use of an ECLIPSE E800 immunofluorescence microscope (Nikon, Tokyo, Japan). Where indicated, an LSM 410 confocal laser scanning microscope (Carl Zeiss, Oberkochen, Germany) was used. Assay for Internalization of transferrin—Assay for internalization of transferrin in HeLa cells or KB cells was performed as described (24). Briefly, HeLa cells or KB cells were transfected with the plasmids encoding the indicated proteins by use of Superfect reagent (Qiagen). After 18 h, the cells were incubated at 37 °C for 1 h with serum-free DMEM, followed by incubation at 37 °C for 1 h with serum-free DMEM containing 0.1 mg/ml Texas Red-conjugated transferrin (Molecular Probes, Inc.). The cells were then immunostained and analyzed. Assay for Myosin Phosphorylation—Cell Migration—A rat brain cDNA library in Prokaryotic and eukaryotic expression vectors were constructed in pMALC2 (New England Biolabs), pGEX (Amersham Pharmacia Biotech), pRSET (Invitrogen), and pEGFP (CLONTECH) by use of standard molecular biology protocols. Full-length rabphilin-11 was constructed in the pRSET vector. Full-length His6-rabphilin-11 was produced in vitro and labeled with [35S]methionine by use of the TNT T7-coupled reticulocyte lysate system (Promega). The truncated mutants of rabphilin-11 were constructed in the pMALC2 and pGEX vectors as follows: pMALC2-rabphilin-11–1, aa 1–631; pMALC2-rabphilin-11–2, aa 632–908; pMALC2-rabphilin-11–3, aa 53–301; and pGEX-rabphilin-11–3, aa 53–301. The full-length and truncated mutants of rabphilin-11 were constructed in the pEGFP vector; pEGFP-rabphilin-11, full length; pEGFP-rabphilin-11–1, aa 1–631; and pEGFP-rabphilin-11–4, aa 667–730. Cell Culture and Transfection—MDCK cells were supplied by Dr. W. Birchmeier (Max-Debruck-Center for Molecular Medicine, Berlin, Germany). HeLa cells were supplied by Dr. S. Orita (Discovery Research Laboratory, Shionogi & Co. Ltd., Osaka, Japan). KB cells were supplied by Dr. Y. Miyata (Tokyo Metropolitan Institute of Medical Science, Tokyo, Japan). MDCK cells were cultured at 37 °C in a humidified atmosphere of 10% CO2 and 95% air in DMEM containing 10% FCS (Life Technologies, Inc.), 100 units/ml penicillin, and 100 g/ml streptomycin. HeLa cells and KB cells were cultured at 37 °C in a humidified atmosphere of 10% CO2 and 95% air in DMEM containing 10% FCS (Life Technologies, Inc.), 100 units/ml penicillin, and 100 μg/ml streptomycin. HeLa cells were cultured on the dishes precoated with fibronectin. Fibronectin-coated dishes were prepared by incubation of non-treated dishes with 10 μg/ml human plasma fibronectin (Sigma) at 4 °C overnight, followed by incubation with 1% BSA/PBS at 37 °C for 1 h. HeLa cells, which had been cultured on non-treated dishes, were trypsinized and washed once in serum-free DMEM. The cells were resuspended in serum-free DMEM (2 × 104 cells/ml), placed on fibronectin-coated dishes, and used for experiments. Transient transfection of pEGFP-rabphilin-11 into MDCK cells or HeLa cells was performed by use of Superfect reagent (Qiagen). MDCK cells stably expressing a Myc-tagged dominant active mutant of Rab11 (sMDCK-Myc-Rab11Q70L cells) or a Myc-tagged dominant negative mutant of Rab11 (sMDCK-Myc-Rab11Q70N cells) were established (23). Stable transfection of pSro-Myc-Rab11Q70L in HeLa cells (sHeLa–Myc-Rab11Q70L cells) was carried out by use of Superfect reagent (Qiagen), and cell clones were isolated by resistance to 4G18 as described previously (23). sMDCK cells were maintained at 37 °C in a humidified atmosphere of 10% CO2 and 95% air in DMEM containing 10% FCS (Life Technologies, Inc.), 100 units/ml penicillin, 100 μg/ml streptomycin, and 0.75 mg/ml G418. sHeLa cells were maintained at 37 °C in a humidified atmosphere of 5% CO2 and 95% air in the same medium. RESULTS Identification, Partial Purification, and Characterization of Rabphilin-11—We identified a band of [35S]GTPyS-GST-Rab11-binding activity with a Mr of about 140,000 in the cytosolic fraction of bovine brain by use of a blot overlay method with [35S]GTPyS-GST-Rab11 (data not shown). We highly purified this protein (p140) from the cytosolic fraction of bovine brain by successive chromatographies of Mono Q, hydroxyapatite, Mono Q, and phenyl-5PW RP columns as described under “Experimental Procedures.” On the final phenyl-5PW RP column chromatography, the [35S]GTPyS-GST-Rab11-binding protein band coincided with a protein with a Mr of about 140,000 which was identified by protein staining (Fig. 1, A—C). p140 bound [35S]GTPyS-GST-Rab11 more preferentially than...
After we had submitted this original manuscript, Zeng et al. isolated a putative target of Rab11 from bovine brain by the method similar to ours, determined its nucleotide and deduced aa sequences, and named it Rab11BP (Rab11-binding protein). All the partial aa sequences of our p140 were included in the deduced aa sequence of Rab11BP. Comparison of deduced aa sequences of our rat and their bovine proteins revealed that they showed 85% identity over the entire sequences (data not shown). This aa difference might be due to the differences in species, and our rabphilin-11 is most likely to be a rat counterpart of bovine Rab11BP.

**Characterization of Recombinant Rabphilin-11**—By use of recombinant rat rabphilin-11, we found that \[^{35}\text{S}\]GTP\_S\_GST-Rab11 binding activity was located in the N-terminal fragment (aa 1–631) and not in the C-terminal fragment (aa 632–908) (Fig. 2Ab). This N-terminal fragment, including the GTP-Rab11 binding domain, bound \[^{35}\text{S}\]GTP\_S\_GST-Rab11 more preferentially than \[^{35}\text{S}\]GDP\_S\_GST-Rab11 (Fig. 2Ab). The N-terminal fragment did not stimulate the GTPase activity of Rab11 (data not shown). The rat brain cytosol (50 μg of protein) or MBP-rabphilin-11–1 (0.2 μg of protein) was subjected to SDS-PAGE, followed by \[^{35}\text{S}\]GTP\_S\_GST-Rab11 blot overlay. a, the rat brain cytosol; b, MBP-rabphilin-11–1. Lower molecular mass bands seen in A, b, lane 1, may be degradation products of rabphilin-11. B, small G protein specificity of rabphilin-11. The rat brain cytosol (50 μg of protein) was subjected to SDS-PAGE, followed by \[^{35}\text{S}\]GTP\_S\_GST-Rab11 blot overlay in the presence of 0.65 μM various nonradioactive small G proteins (130-fold higher concentration than that of \[^{35}\text{S}\]GTP\_S\_GST-Rab11). a, the rat brain cytosol; b, MBP-rabphilin-11–1. Lane 1, \[^{35}\text{S}\]GTP\_S\_GST-Rab11 alone; lane 2, in the presence of nonradioactive GTP\_S\_GST-Rab11; lane 3, in the presence of nonradioactive GTP\_S\_GST-Rab3a; lane 4, in the presence of nonradioactive GTP\_S\_GST-Rab5; lane 5, in the presence of nonradioactive GTP\_S\_GST-RhoA.

**Tissue Distribution of Rabphilin-11**—Rab11 is expressed in various tissues but abundant in brain, lung, adrenal gland, kidney, pancreas, and testis (6, 30). Northern blot analysis indicated that rabphilin-11 was also expressed in various rat tissues examined (Fig. 4). Of various cell lines, rabphilin-11 was abundant in MDCK cells, HeLa cells, and PC12 cells (data not shown).
endogenous rabphilin-11 was stained in MDCK cells stably expressing Myc-Rab11Q70L (sMDCK-Myc-Rab11Q70L cells) and sMDCK-Myc-Rab11S25N cells, essentially the same results were obtained (data not shown). These results suggest that rabphilin-11 binds GTP-Rab11 not only in a cell-free system but also in intact cells and that they are colocalized at perinuclear regions, which presumably correspond to the Golgi complex and recycling endosomes.

Colocalization of Rabphilin-11 with GTP-Rab11 along Microtubules—It has been shown that microtubules are oriented toward lamellipodia (31) and that microtubules are involved in vesicle recycling (32–34). We examined the localization of a Myc-tagged dominant active mutant of Rab11 (Myc-Rab11Q70L) and rabphilin-11 in HeLa cells cultured on fibronectin. The HeLa cells cultured on fibronectin showed well developed lamellipodia toward which microtubules were oriented (31) and that microtubules are involved in vesicle recycling (32–34). We examined the localization of a Myc-tagged dominant active mutant of Rab11 (Myc-Rab11Q70L) and rabphilin-11 in HeLa cells cultured on fibronectin. The HeLa cells cultured on fibronectin showed well developed lamellipodia toward which microtubules were oriented (31) and that microtubules are involved in vesicle recycling (32–34). 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that both the proteins are localized presumably on the recycling vesicles at perinuclear regions and along microtubules.

Involvement of Rabphilin-11 in Accumulation of Transferrin at Perinuclear Regions—We examined whether rabphilin-11 indeed regulates vesicle recycling as described for Rab11 (9, 11). Overexpression of a Myc-tagged dominant negative mutant of Rab11 (Myc-Rab11S25N) in HeLa cells markedly inhibited accumulation of transferrin at perinuclear regions as compared with those in wild-type cells (data not shown), consistent with the earlier observations (9, 11). Similarly, overexpression of the GFP-tagged C-terminal fragment of rabphilin-11 (GFP-rabphilin-11–4, aa 607–730), lacking the GTP-Rab11 binding domain, markedly inhibited this accumulation (Fig. 7A, a and b). However, overexpression of GFP-tagged full-length rabphilin-11 did not affect this accumulation (Fig. 7A, c and d). Overexpression of the GFP-tagged N-terminal fragment of rabphilin-11 (GFP-rabphilin-11–1, aa 1–631), including the GTP-Rab11 binding domain, did not affect this accumulation, either (data not shown). The essentially similar results were obtained when the similar experiments were done with KB cells (Fig. 6).
It is not clear to which intracellular organelles the perinuclear regions correspond, but they may be the Golgi complex and recycling endosomes. These results, together with the earlier observations that Rab11 regulates vesicle recycling (9, 11), suggest that rabphilin-11 also regulates vesicle recycling.

Transferrin was also observed as small dots widely distributed throughout the cytoplasm in HeLa cells and KB cells (Fig. 7). It is not clear where these dots correspond, but they may be early endosomes. If this is the case, Rab11 and rabphilin-11 may not be involved in vesicle trafficking from the plasma membrane to early endosomes by endocytosis, and this conclusion is consistent with the earlier observations for Rab11 (9, 11).

It has been reported by Zeng et al. (29) that the N-terminal fragment of Rab11BP (aa 1–504) inhibits the accumulation of transferrin at endosomes in TRVb cells, and our above result is apparently inconsistent with this observation. The exact reason for this discrepancy is not known at present, but this might be due either to different lengths of the N-terminal fragments of Rab11BP and rabphilin-11 or to a lower expression level of the N-terminal fragment of rabphilin-11 (aa 1–631) in our assay system than that of the N-terminal fragment of Rab11BP (aa 1–504) in their assay system. They did not examine the effect of the C-terminal fragment of Rab11BP, but our results clearly indicate that the C-terminal fragment of rabphilin-11 (aa 607–730) reduces the accumulation of transferrin at perinuclear regions (Fig. 7, Aa and Ba), and the expression levels of the N-terminal and C-terminal fragments were apparently similar (data not shown). Therefore, it could be concluded from their experiments and our experiments that the C-terminal fragment might be more effective than the N-terminal fragment on inhibiting vesicle recycling.

**Involvement of Rabphilin-11 in Cell Migration** It has been shown that vesicle recycling is necessary for cell migration (35, 36). Finally, we examined whether Rab11 and rabphilin-11 are involved in cell migration. For this purpose, we first used MDCK cells stably expressing a Myc-tagged dominant active mutant of Rab11 (sMDCK-Myc-Rab11Q70L cells) or MDCK cells stably expressing a Myc-tagged dominant negative mutant of Rab11 (sMDCK-Myc-Rab11S25N cells), and we esti-
mated cell migration by use of the gold particle uptake assay. Wild-type MDCK cells and sMDCK-Myc-Rab11Q70L cells migrated to a similar extent, but sMDCK-Myc-Rab11S25N cells migrated significantly to a lesser extent (data not shown). Because MDCK cells stably expressing various mutants of rabphilin-11 were not available, we transiently overexpressed GFP-tagged full-length rabphilin-11, the GFP-tagged N-terminal fragment (GFP-rabphilin-11–1, aa 1–631), or the GFP-tagged C-terminal fragment of rabphilin-11 (GFP-rabphilin-11–4, aa 607–730) in HeLa cells cultured on fibronectin, and we assayed their migration. Overexpression of the C-terminal fragment, but not full-length rabphilin-11 or the N-terminal fragment, markedly reduced cell migration (Fig. 8). These results suggest that both Rab11 and rabphilin-11 are involved in cell migration. We have not examined here the overexpression of Rab11S25N in HeLa cells, because GFP-tagged Rab11S25N was not available, but the inhibitory effect of Rab11S25N on migration of MDCK cells is consistent with the inhibitory effect of the C-terminal fragment of rabphilin-11 on migration of HeLa cells.

**DISCUSSION**

In this study, we have isolated and characterized a GTP-Rab11-binding protein and named it rabphilin-11. We have concluded that rabphilin-11 is a downstream target of Rab11 that has been implicated in vesicle recycling (8–11) on the basis of the following observations: 1) it binds GTP-Rab11 more preferentially than GDP-Rab11 without stimulating the GTPase activity of Rab11; 2) it is colocalized with GTP-Rab11 at perinuclear regions in MDCK cells and HeLa cells and also along microtubules in HeLa cells; and 3) rabphilin-11 as well as Rab11 regulates accumulation of transferrin at perinuclear regions and cell migration.

We have shown here that Rab11 and rabphilin-11 are colocalized at perinuclear regions in HeLa cells and KB cells and that both the proteins regulate the accumulation of transferrin there without affecting its accumulation at small dots which are widely distributed in the cytoplasm. The precise localization sites of GTP-Rab11 and rabphilin-11 at perinuclear regions are not known, but they are likely the Golgi complex and recycling endosomes, and the small dots in the cytoplasm may be early endosomes. If these sites are correct, both the proteins may regulate at least vesicle recycling from early endosomes to recycling endosomes.

Moreover, we have shown here that Rab11 and rabphilin-11 are colocalized along microtubules oriented toward lamellipodia. Microtubules are known to be implicated in transport from early endosomes to late endosomes and apical vesicle recycling, both of which are inhibited by nocodazole in polarized MDCK cells (33, 34). It is not known whether Rab11 and rabphilin-11 are involved in apical vesicle recycling along microtubules, but the localization of Rab11 and rabphilin-11 along microtubules oriented toward lamellipodia has raised their possible involvement in apical vesicle recycling.

Vesicle recycling of various transmembrane proteins, including adhesion molecules such as integrin, has been implicated in cell migration (37–40). We have recently shown that dynamic rearrangement of focal adhesion and stress fibers are observed during migration of MDCK cells and that Rab GDI, a general regulator of all the Rab family members, and at least Rab5 are involved in this rearrangement, most presumably by regulating endocytosis and recycling of integrin (23). Consistently, we have shown here that overexpression of a dominant negative mutant of Rab11 (Rab11S25N) and the C-terminal fragment of rabphilin-11 (aa 607–730), lacking the GTP-Rab11 binding domain, reduce migration of MDCK cells and HeLa cells, respectively. It remains to be clarified how these proteins reduce cell migration, but they may reduce cell migration by inhibiting the vesicle recycling from early endosomes to recycling endosomes. Of the downstream targets of the Rab family members thus far reported, Shirataki et al. (41) and Mckiernan et al. (42) have shown that rabphilin-3, a target of Rab3, is associated with synaptic vesicles in a manner independent of GTP-Rab3A, although Stahl et al. (43) have shown that it is associated with the vesicles in a manner dependent on GTP-Rab3A, whereas rabaptin-5, a target of Rab5, is associated with early endosomes in a manner dependent on GTP-Rab5 (15). We have shown here that both GDP-Rab11 (a Myc-tagged dominant negative mutant of Rab11, Myc-Rab11S25N) and rabphilin-11 are found in the cytoplasm in MDCK cells stably expressing Myc-Rab11S25N (sMDCK-Myc-Rab11S25N cells), whereas both GTP-Rab11 (a Myc-tagged dominant active mutant of Rab11, Myc-Rab11Q70L and rabphilin-11 are colocalized at perinuclear regions in MDCK cells stably expressing Myc-Rab11Q70L (sMDCK-Myc-Rab11Q70L cells). These results suggest that rabphilin-11 is translocated between the cytoplasm and the membranes of perinuclear regions coupled with the cycling of Rab11 between the GDP-bound and GTP-bound forms.

In conclusion, rabphilin-11 is a downstream target of Rab11 that regulates vesicle recycling. Further studies are necessary for our understanding of the modes of activation and action of Rab11 and rabphilin-11 in vesicle recycling.

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