Plasma membrane recycling is an essential process in the maintenance and regulation of the normal complement of membrane pumps, channels, and receptors in mammalian cells. Although the handling of individual cargo proteins may vary among cell types, a number of studies over the past several years have established a central role for the small GTPases Rab11a and Rab25 in plasma membrane recycling. In non-polarized cells, transferrin receptor recycling through a perinuclear recycling system dependent on Rab11a (1–3), Rab25 is specifically expressed in epithelial cells (4). In polarized MDCK cells, Rab11a and Rab25 regulate both transcytosis and apical recycling of the polymeric IgA receptor (pIgA-R) through the apical recycling system (5, 6).

Recently, several groups have reported proteins that interact with Rab11a and its related family members Rab11b and Rab25. Rab11-binding protein Rabphilin 11 associates with GTP-bound Rab11a and colocalizes with Rab11a (7, 8). The pp75/Rab11 interacting protein (Rip11) localized with Myc-tagged Rab11a in rat kidney cells and associated with IgA trafficking in MDCK cells (9). The pp75/Rip11 protein is a member of a larger group of Rab11 family interacting proteins (Rab11-FIPs). Rab11-FIP1, Rab11-FIP2, Rab11-FIP3, Rab11-FIP4, Rab coupling protein (RCP), and pp75/Rip11 all interact with Rab11a through a conserved carboxyl-terminal amphipathic α-helical domain (10–13). McCaffrey and colleagues (13) have also reported that RCP can interact with Rab4. In addition to the Rab11 binding region, Rab11-FIP2, RCP, and pp75/Rip11 all contain an amino-terminal C2 domain (10–13). In parietal cells, MDCK cells, and HeLa cells, multiple Rab11-FIP proteins are present spatially within populations of Rab11a-containing recycling vesicles (10).

Finally, we have also reported that the actin motor protein myosin Vb associates specifically with Rab11 family GTPases (14). Myosin Vb immunoreactivity was present on Rab11a-containing recycling vesicles and GFF-myosin Vb targeted to recycling system vesicles in both HeLa and MDCK cells (14). Importantly, the overexpression of the tail of myosin Vb lacking a motor domain caused accumulation of Rab11a in pericentriolar vesicle aggregates and strongly inhibited trafficking out of the recycling system in both HeLa and MDCK cells (14). Thus, GFF-myosin Vb transfection blocked exit of transferrin from the recycling system vesicles in HeLa cells and pIgA-R from the apical recycling system in MDCK cells. All of these results indicated that myosin Vb was the motor protein responsible for transit out of plasma membrane recycling systems.

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Myosin Vb is a member of the class V myosins (myosin Va, myosin Vb/myr6, and myosin Vc) that promote translocation along the actin cytoskeleton and appear to associate with distinct Rab GTPases. Although the Rab protein partner for myosin Vc remains obscure (15), recent investigations have demonstrated the association of myosin Va with vesicles containing Rab27a (17). Myosin Va organizes the distribution and transport of Rab27a-containing melanosomes in melanocytes (16, 17). Mutations in Rab27a or myosin Va lead to neurological abnormalities and the inability to recruit melanosomes in both rodent models and humans (18–20). The bond between Rab27a

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1 The abbreviations used are: MDCK, Madin-Darby canine kidney; pIgA-R, polymeric IgA receptor; Rab11-FIP, Rab11 family interacting protein; RCP, Rab coupling protein; GFP, green fluorescent protein; GST, glutathione S-transferase.
and myosin Va is indirect (17), and recent efforts have indicated that melanophilin/Slp homologue lacking C2 domains (Slac2-a) functions as the receptor recruited by Rab27a for myosin Va binding (21–23).

Rab11-FIP2 localized with Rab11a in MDCK and HeLa cells and associated with Rab11a in yeast two hybrid assays (10). In vitro, [γ-32P]GTP Rab11a bound directly to recombinant Rab11-FIP2 (10). Rab11-FIP2 co-enriched with Rab11a in rabbit gastric membrane fractions and translocated with Rab11a to the canaliculi in stimulated parietal cells (10). Recent studies from McCreery and colleagues (12) have suggested that Rab11-FIP2 can dimerize and may also form hetero-oligomers with other Rab11-FIP family members. Importantly, our original yeast two hybrid studies indicated that Rab11-FIP2 could interact with the tail of myosin Vb (10). We hypothesized that Rab11-FIP2 may contribute to anchoring of Rab11a-positive vesicles to myosin Vb.

We now report that yeast two hybrid analysis, immunolocalization experiments, and in vitro binding studies all confirm the direct association of Rab11-FIP2 with myosin Vb. In trafficking studies, a truncation of Rab11-FIP2 lacking the amino-terminal C2-domain inhibited plasma membrane recycling in both HeLa and MDCK cells. These studies show that Rab11-FIP2 is a receptor/adaptor protein linking myosin Vb to Rab11a and regulating the movement of vesicle cargo through the recycling endosome.

EXPERIMENTAL PROCEDURES

Yeast Two Hybrid—Yeast two hybrid binary assays were conducted as described previously (10). Briefly, truncations of Rab11-FIP2 were amplified by PCR adding an EcoRI site to the 5′ end and a SalI site to the 3′ end. PCR amplification products were then digested with EcoRI and SalI (New England Biolabs) and cloned into pBD-Gal-(CaM) (Stratagene) vector utilizing T4 DNA ligase (New England Biolabs). Plasmids were confirmed with automated sequencing (Molecular Biological Core Facility, Medical College of Georgia). Rab11-FIP2 truncations were then cotransfected with either pAD-myosin Vb tail or pAD empty vector as a control into the yeast strain YI90. Yeast were allowed to grow for 3 days at 30 °C and then analyzed via β-galactosidase assay. A positive result represented blue color within 4 h.

GST Association Assays—Recombinant GST-myosin Vb tail attached to glutathione-Sepharose beads and recombinant His-Rab11-FIP2, His-Rab11-FIP2 (129–356), His-Rab11-FIP2 (129–290), and His-Rab11-FIP2 (191–290) were made as described previously (10). Myosin Vb tail was amplified with SalI sites added to both ends. The sample was cut with SalI, cloned into pGEX5x-1, and confirmed for sequence accuracy. Myosin Vb tail in pGEX5x-1 and pGEX5x-1 empty vector were transfected into BL21LysS competent cells (Promega), and recombinant protein was produced following induction with isopropyl-1-thio-β-D-galactopyranoside (1 mM) for 3 h. GST and GST-myosin Vb tail beads were stored in phosphate-buffered saline with protease inhibitor mixture (Sigma) at 4 °C. Rab11-FIP2 and Rab11-FIP2 truncations were PCR amplified and cloned into pET30a (Novagen) for His-tagged recombinant protein production.

For a single pull-down reaction, 50 µl of GST and GST-myosin Vb tail beads were blocked for 2 h in buffer then digested with EcoRI and SalI (New England Biolabs) and cloned into pBD-Gal-(CaM) (Stratagene) vector utilizing T4 DNA ligase (New England Biolabs). Recombinant GST-myosin Vb tail attached to glutathione-Sepharose beads were stored in phosphate-buffered saline with protease inhibitor mixture (Sigma) at 4 °C. Rab11-FIP2 and Rab11-FIP2 truncations were PCR amplified and cloned into pET30a (Novagen) for His-tagged recombinant protein production.

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co-localized with both GFP-myosin Vb tail and endogenous Rab11a (Fig. 1). The results suggest that all of these Rab11 interacting proteins may function in a contiguous vesicle recycling system ultimately dependent on myosin Vb activity.

**Myosin Vb Tail Binding Site in Rab11-FIP2 Is Not Located within the Rab11 Binding Site or the Putative C2 Domain—**Because yeast two hybrid data has indicated an association between myosin Vb and Rab11-FIP2 (10), further yeast two hybrid assays were used to determine the myosin Vb binding site in Rab11-FIP2. Amino acids 129–356 of Rab11-FIP2 (Rab11-FIP2 (129–356)) were sufficient to maintain an association with myosin Vb tail (Fig. 2). Interestingly, when truncated from the carboxyl terminus to amino acid 465, Rab11-FIP2 would associate nonspecifically. Truncation analysis indicated that the autoactivation domain mapped to amino acids 429–465 in Rab11-FIP2 (data not shown). Similar yeast two hybrid assays were conducted in which full-length Rab11-FIP2 was tested against deletion mutants of myosin Vb tail. Deletion of either 45 amino acids of the amino terminus (myosin Vb tail (45–588)) or 32 amino acids of the carboxyl terminus (myosin Vb tail (1–553)) of the myosin Vb tail eliminated interaction with Rab11-FIP2. In contrast, we have demonstrated previously that myosin Vb tail (45–588) and myosin Vb tail (1–553) could interact with Rab11a in yeast two hybrid studies (14).

**GST-Rab11-FIP2 Binds to Myosin Vb in Vitro—**To confirm the myosin Vb tail/Rab11-FIP2 association, GST fusion protein binding assays were used. Rab11-FIP2 strongly associated with GST-myosin Vb tail beads compared with little detectable interaction with GST control beads (Fig. 3). Despite the presence of protease inhibitors, His-Rab11-FIP2 was highly susceptible to proteolysis especially in supernatant fractions (data not shown). In agreement with yeast two hybrid results, Rab11-FIP2 (129–356) was also retained on GST-myosin Vb tail beads, compared with no detectable association with GST beads. (Fig. 3). Interestingly, GST binding assays indicated an association between myosin Vb tail and Rab11-FIP2 (129–290) but not the smaller fragment Rab11-FIP2 (191–290) thus narrowing the myosin Vb tail binding region.

**In Situ Confirmation of Myosin Vb Interaction with Rab11-FIP2 Using Dual Transfection—**To confirm the association of myosin Vb and Rab11-FIP2 in situ, we studied the distribution of GFP-Rab11-FIP2 truncations with co-expressed DsRed-myosin Vb tail. In singly transfected MDCK cells, GFP-Rab11-FIP2 was distributed in a multi-vesiculated pattern similar to that observed for immunostaining of endogenous Rab11-FIP2 (Fig. 4A). However, GFP-Rab11-FIP2 (129–512), which lacked the amino-terminal C2 region of Rab11-FIP2, was distributed to a more compact large punctate spot (Fig. 4A). Other GFP-Rab11-FIP2 truncations, which did not contain the carboxy-terminal Rab11 binding site, demonstrated a predominantly
cytosolic distribution (Fig. 4A). In MDCK cells (Fig. 4B), both GFP-Rab11-FIP2 and GFP-Rab11-FIP2 (129–512) colocalized with co-expressed DsRed-myosin Vb tail. As a control, non-chimeric GFP alone did not colocalize with DsRed-myosin Vb tail (data not shown). Importantly, compared with their cytosolic distribution in single transfection studies (Fig. 4A), GFP-Rab11-FIP2 (1–465), GFP-Rab11-FIP (129–356), and GFP-Rab11-FIP2 (129–290) colocalized with DsRed-myosin Vb tail in a punctate pattern. Identical results were observed with dual transfections into HeLa cells (data not shown). These studies confirmed the in vitro association assays.

**Rab11-FIP2 Regulates Apical Recycling and Transcytosis of IgA**—In MDCK cells stably expressing pIgA-R, apical recycling or transcytosis of IgA is trafficked through Rab11a-containing vesicles (5). GFP-myosin Vb tail concentrates pIgA-R and disrupts IgA transcytosis (14). Thus, we sought to determine the involvement of Rab11-FIP2 in IgA trafficking. GFP-Rab11-FIP2 (129–512) was chosen for the assay, because it elicited a tight punctate distribution similar to the dominant negative acting myosin Vb tail (Fig. 4A). A tetracycline repressible line containing GFP-Rab11-FIP2 (129–512) was created to compare trafficking in cells in the absence or presence of overexpressed GFP-Rab11-FIP2 (129–512). Fig. 5 demonstrates that polymeric IgA-R co-localized with both GFP-Rab11-FIP2 (129–512) and Rab11a in concentrated intracellular puncta in each cell. Western blots of whole cell extracts from cells grown in the presence or absence of doxycycline indicated undetectable expression levels of GFP-Rab11-FIP2 (129–512) in the presence of 5 ng/ml antibiotic (Fig. 6B, inset).

Conventional IgA trafficking assays have utilized 125I-IgA to quantify transcytosed or recycling IgA (5); however we chose to develop a fluorescent ligand-based assay. Alexa 546-IgA was utilized as the cargo in both apical recycling and basolateral to apical transcytosis assays. In the absence of doxycycline, GFP-Rab11-FIP2 (129–512) expressing cells demonstrated a significant decrement in both IgA apical recycling and transcytosis (Fig. 6). At the 40-min time point for IgA apical recycling and at the 60-min time point for IgA transcytosis, GFP-Rab11-FIP2 (129–512) expressing cells accumulated the fluorescent Alexa 546-IgA cargo in the apical portion of the cells (Fig. 6A). In both cases, Alexa 546-IgA was accumulated in GFP-Rab11-FIP2 (129–512) containing vesicle aggregates. In the presence of doxycycline, IgA apical recycling and transcytosis occurred normally. Alexa 546-IgA trafficked out of the cells and did not accumulate at the 40- and 60-min time points for apical recycling and transcytosis, respectively (Fig. 6A). GFP-Rab11-FIP2 (129–512) expressing cells demonstrated 33% higher retention.
of transcytosing IgA compared with cells not expressing the chimera (Fig. 6B). Similarly, 31% more apically recycling Alexa 546-IgA was retained in GFP-Rab11-FIP2 (129–512) expressing cells compared with cells maintained in the presence of doxycycline (Fig. 6C). The results suggest that GFP-Rab11-FIP2 (129–512), like GFP-myosin Vb tail, acts as a potent inhibitor of IgA trafficking in polarized MDCK cells.

Rab11-FIP2 (129–512) Disrupts the Recycling of Transferrin in Non-polarized HeLa Cells—In non-polarized HeLa cells, transferrin cycles through Rab11a-containing vesicles. Previous studies have shown that the overexpression of either the dominant-negative mutant Rab11aS25N (1) or the GFP-myosin Vb tail (14) inhibited normal transferrin recycling. Recent findings (26) have indicated that truncations at the amino terminus of Rab11-FIP2 may delay the cycling of transferrin causing accumulation of transferrin in tubular type structures. We determined the effect of GFP-Rab11-FIP2 (129–512) on transferrin trafficking in transiently transfected HeLa cells. At the 0-time point, GFP-Rab11-FIP2 (129–512)-transfected cells accumulated Alexa 546-transferrin into a central perinuclear spot, whereas in non-transfected cells Alexa 546-transferrin maintained the normal more dispersed perinuclear distribution of the recycling system (Fig. 7A). The distribution of transferrin receptor immunostaining co-localized with fluorescent transferrin in both non-transfected and transfected cells (Fig. 7A). After 40 min of serum chase, GFP-Rab11-FIP2 (129–512)-transfected cells still retained the accumulated Alexa 546-transferrin, whereas non-transfected cells had recycled the Alexa 546-transferrin (Fig. 7). Thus, whereas non-transfected and transfected cells contained similar amounts of transferrin after loading, transfected cells still retained over 70% of the fluorescent transferrin after 40 min of serum chase (Fig. 7B). In contrast, less than 1% of the transferrin was retained in non-transfected cells (Fig. 7B).

DISCUSSION

Movement of vesicles along cytoskeletal elements requires targeting of specific vesicle systems to appropriate pathways. Recent studies have demonstrated that Rab GTPases regulate many aspects of this targeting. Rab6 interacts with a specific kinesin isoform, rabkinesin 6, and regulates trafficking through the Golgi apparatus (27, 28). Rab27a targets myosin...
Va to melanosomes for regulation of organelle distribution in melanocytes (16–17). Similarly, we have demonstrated that Rab11a is a receptor for myosin Vb on the vesicles of the plasma membrane recycling system (14). Although the interaction of myosin Va with Rab27a is clearly indirect and mediated by a linker protein (21–23), melanophilin/Slac2-a, we have provided evidence previously for a direct interaction of Rab11a with myosin Vb tail (14). The present investigations demonstrate that Rab11-FIP2 acts as a coordinator of the interaction of Rab11a with myosin Vb by binding to both Rab11a and myosin Vb.

All three members of the mammalian class V myosins appear to interact with distinct vesicle populations within cells. Myosin Va interacts with melanosomes and lytic granules of cytoxic T lymphocytes (17). Targeting of myosin Va to melanosomes is mediated by recruitment of melanophilin/Slac2-a to granules by Rab27a (21–23). Melanophilin can then bind myosin Va thereby bringing melanosomes in contact with the actin-based motor. In a similar fashion, Rab11a appears to recruit both Rab11-FIP2 and myosin Vb to the recycling system vesicles. Although we have demonstrated previously co-distribution of Rab11a and myosin Vb on recycling system vesicles, a direct interaction could only be established in yeast two hybrid assays (14). Rab11a interacts directly with Rab11-FIP2 through a discrete amphipathic helical motif located in its carboxyl terminus (10). In the present studies, we have demonstrated an interaction of Rab11-FIP2 with the tail region of myosin Vb through yeast two hybrid studies, in vitro binding assays, and in situ interaction studies in both HeLa and MDCK cells. All of these studies point to the association of Rab11-FIP2 with myosin Vb through a centrally located domain, which lies between the amino-terminal C2 domain and the carboxyl-terminal Rab11-binding helix. In addition, our studies also demonstrate that the Rab11-FIP2 binding region encompasses the entire myosin Vb tail. Notably, the pattern of interactions observed in yeast two hybrid assays for Rab11-FIP2 with myosin Vb was different from that observed for Rab11a and myosin Vb (14). These results indicate that the binding sites for Rab11a and Rab11-FIP2 are differentiable. Thus, these studies suggest that, in contrast with the pattern observed for Rab27a and myosin Va, Rab11a may recruit directly both its motor protein, myosin Vb, and is coordinating adapter, Rab11-FIP2.

In addition to interacting with myosin V motors, both Rab11a and Rab27a also interact with multi-member families of interacting proteins. Rab27a can interact with at least four proteins with homologous Rab27a binding regions (29). These include melanophilin, synaptotagmin-like proteins 1 and 2-a (Slp1 and Slp2a), and granulophilin. With the exception of melanophilin, all of these proteins have general structural homology with Rabphilin 3, containing amino-terminal Rab binding domains and two tandem C2 domains. Melanophilin lacks the C2 domains and instead contains two coiled regions, the first of which is responsible for interaction with myosin Va (30). Similarly, we and others (10–13) have now identified six members of the Rab11-FIP family: Rab11-FIP1, Rab11-FIP2, Rab11-FIP3, Rab11-FIP4, pp75/Rip11, and RCP. All of these proteins contain a conserved carboxy-terminal Rab binding domain. In addition, Rab11-FIP2, RCP, and pp75/Rip11 contain single amino-terminal C2 binding domains. Although neutral phospholipid binding to pp75/Rip11 has been demonstrated (9), the functions of the C2 domains in Rab11-FIP protein remain obscure.

The present results demonstrate that the C2 domain of Rab11-FIP2 is necessary for proper function of the recycling system. Thus, overexpression of the Rab11-FIP2 truncation lacking the C2 domain strongly inhibited trafficking through recycling systems. It is notable that presence of the carboxy-terminal Rab binding domain was necessary for targeting of Rab11-FIP2 to the apical recycling system. However, in the presence of overexpressed myosin Vb tail, fragments of Rab11-FIP2 containing the myosin Vb binding site could be recruited to the recycling system vesicles. These results suggest that the interaction of myosin Vb and Rab11-FIP2 can occur independent of their interactions with Rab11a. These findings also support the notion that myosin Vb tail can be targeted to the recycling system independent of an interaction with Rab11-FIP2. Indeed, it is notable that, whereas Rab11-FIP2 can interact with Rab11a independent of its guanine nucleotide binding status, myosin Vb tail only interacts with the GTP-bound form of Rab11a (10, 14).

The findings presented in this report demonstrate that overexpression of myosin Vb tail causes concentration of not only Rab11a but also of Rab11-FIP1, Rab11-FIP2, and pp75/Rip11. Because all of these proteins bind to Rab11 family members through the same amphipathic helical motif, this pattern of concentration of three Rab11-FIP proteins by myosin Vb tail suggests that some coordination of proteins must be involved in

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**Fig. 7.** Transferrin trafficking in nonpolarized HeLa cells. A. HeLa cells were transiently transfected with GFP-Rab11-FIP2 (129–512), and Alexa 546-transferrin was loaded and chased with unlabeled transferrin. Cells were fixed at 0 and 40 min and immunostained for human transferrin receptor. White arrows indicate triple colocalization among GFP-Rab11-FIP2 (129–512), Alexa 546 transferrin, and transferrin receptor. White bar in lower left corner equals 5 microns. B, quantitative analysis of transferrin retained at 0 and 40 min after chase (± S.E.). Black bar, GFP-FIP2 (129–512) transfected cells. Gray bar, non-transfected cells. One intensity unit on the chart represents 1000 intensity units from Metamorph pixel intensity quantification. The figure is representative of three separate experiments.
regulating recycling system trafficking. Individual Rab11-FIP proteins may mark specific subdomains within the continuous recycling compartment (31). To some extent this could be regulated by Rab11-FIP protein interaction with either Rab11a or Rab25. Compared with Rab11a, Rab25 has an opposite and inhibitory influence on trafficking through the apical recycling system or diversion of Rab11-FIP proteins. It is also possible that Rab11-FIP proteins are recruited to recycling system vesicles by Rab11a to be assembled into multimeric complexes. MacCaffrey and colleagues (12) have reported that Rab11-FIP proteins can homo- and heterodimerize. We have also observed the homodimerization of FIP2 in yeast two hybrid assays. This homodimerization is dependent on the C2 domain of Rab11-FIP2. Thus, it is possible that the inhibitory effects of GFP-FIP2 (129–512) may result from a loss of the dimerization of Rab11-FIP2.

In summary, we have provided evidence that Rab11-FIP2 is a coordinate adapter for a ternary complex with both Rab11a and myosin Vb. The assembly of multimeric complexes of regulatory Rab11-FIP proteins and the class V myosin motor, myosin Vb, represents a critical step for the proper functioning of plasma membrane recycling systems in both non-polarized and polarized cells.

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REFERENCES

1. Ullrich, O., Reinsch, S., Urbe, S., Zerial, M., and Parton, R. G. (1996) J. Cell Biol. 135, 913–924
2. Green, E. G., Ram, E., Riley, N. M., Spiro, D. J., Goldenring, J. R., and Wessling-Resnick, M. (1997) Biochem. Biophys. Res. Commun. 239, 612–616
3. Ren, X., Xu, G., Zeng, J., De Lemos-Chiarandini, C., Adesnik, M., and Sabatini, D. D. (1998) Proc. Natl. Acad. Sci. U. S. A. 95, 6187–6192
4. Goldenring, J. R., Shen, K. R., Vaughan, H. D., and Modlin, I. M. (1993) J. Biol. Chem. 268, 18419–18422
5. Casanova, J. E., Wang, X., Kumar, R., Bhartur, S. G., Navarre, J., Woodrum, J. E., Ray, G. S., and Goldenring, J. R. (1999) Mol. Biol. Cell. 10, 47–61
6. Wang, X., Kumar, R., Navarre, J., Casanova, J. E., and Goldenring, J. R. (2000) J. Biol. Chem. 275, 29138–29146

2 C. M. Hales and J. R. Goldenring, unpublished results.
Rab11 Family Interacting Protein 2 Associates with Myosin Vb and Regulates Plasma Membrane Recycling
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