Role of the FYVE Finger and the RUN Domain for the Subcellular Localization of Rabip4*

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Rabip4 is a Rab4 effector, which possesses a RUN domain, two coiled-coil domains, and a FYVE finger. It is associated with the early endosomes and leads, in concert with Rab4, to the enlargement of endosomes, resulting in the fusion of sorting and recycling endosomes. Our goal was to characterize the role of these various domains in Rabip4 subcellular localization and their function in Chinese hamster ovary cells. Although the FYVE finger domain specifically bound phosphatidylinositol 3-phosphate and was necessary for the function of Rabip4, it was not sufficient for the protein association with membranes. Indeed a protein containing the FYVE finger and the Rab4-binding site was cytosolic, whereas the total protein was mostly associated to the membrane fraction, whether or not cells were pre-treated with wortmannin. By contrast, a construct corresponding to the N-terminal end, Rabip4-(1–212), and containing the RUN domain was membrane-associated. The complete protein partitioned between the Triton X-100-insoluble and -soluble fractions and a wortmannin treatment increased the amount of the protein in the Triton X-100 fraction. Rabip4-(1–212) was totally Triton X-100-insoluble, and confocal microscopic examination showed that it labeled not only the early endosomes, positive for Rabip4, but also a filamentous network with a honeycomb appearance. The Triton X-100-insoluble fraction that contains Rabip4 did not correspond to the caveolin or glycosylphosphatidylinositol-enriched lipid rafts. Rabip4 did not appear directly linked to actin but seemed associated to the actin network. We propose that the subcellular localization of the protein is primarily driven by the RUN domain to endosomal microdomains characterized by Triton X-100 insolubility and that the FYVE domain and the Rab4-binding domain then allow for the recruitment of the protein to lipophlic microdomains enriched in phosphatidylinositol 3-phosphate.

Rabip4 (Rab 4- interacting protein) was identified as an effector of Rab4, a small GTPase that plays a role in the endocytic pathway in all cell types (1). In CHO cells, Rabip4 appears as an early endosomal protein, colocalized with early endosome antigen 1 (EEA1), an effector of Rab5 enriched in early sorting endosomes (2, 3). It is absent from the recycling and late endosomes. The coexpression of Rabip4 with Rab4 leads to an enlargement of early endosomes, and thus Rabip4 seems involved in early endosomal traffic (1).

Rabip4 is a 600-amino acid protein, in which we noted the presence of two coiled-coil motifs (4) and a C-terminal FYVE domain (for Fab1p, YOTB, Vac1p, EEA1) finger (5–7). FYVE finger domains are present in a group of proteins such as EEA1 (8), Fab1p (9), Vac1p (10), and Vps27p (10), which are characterized by an endosomal localization. This cysteine-rich domain specifically binds PtdIns(3)P (6, 12, 13), a property that is important for the intracellular localization of those proteins. Indeed, using a double FYVE finger construct, Gillooly et al. (14) very elegantly showed an enrichment of PtdIns(3)P on the surface of early endosomes and in the internal vesicles of multivesicular endosomes. In agreement with the association of EEA1 with endosomes through the binding of its FYVE finger to the PtdIns(3)P, a mutation in this domain causes the redistribution of EEA1 to the cytosol (15). However, the endosomal localization of EEA1 was also determined by its ability to bind Rab5 (16). Hrs is another extensively studied FYVE-containing protein, implicated in both membrane trafficking and signal transduction (17, 18). It presents an endosomal localization, but results are more conflicting about the exact role of its FYVE finger in this localization (5). In conclusion as reviewed in (5), there are no reports of a FYVE finger alone being sufficient to target a protein to a membrane, and additional interactions might be required.

We also reported that the N-terminal part of Rabip4 presents 40% analogy with RPIP8 (Rap2-interacting protein 8) (19) and with the two KIAA 0871 and 1537 (1) and data not shown). These proteins have been shown very recently to contain a RUN domain (for RPIP8, UNC-14, and NESCA) (20). The RUN domain is organized into six conserved blocks, which are predicted to constitute the “core” of a globular structure. Although the role of this RUN domain is not known yet, it appears in several proteins that are particularly linked to the functions of GTPases of the Rap and Rab families (20).

The purpose of this work was to clarify the roles of the FYVE and RUN domains present in Rabip4. We found that the FYVE finger of Rabip4, although it specifically binds PtdIns(3)P in vitro, was insufficient for the endosomal localization of the protein. Furthermore, the N-terminal part containing the RUN

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1 The abbreviations used are: CHO, Chinese hamster ovary; EEA1, early endosome antigen 1; GFP, green fluorescent protein; GPI, glycosylphosphatidylinositol; PtdIns(3)P, phosphatidylinositol 3-phosphate; PAGE, polyacrylamide gel electrophoresis; GST, glutathione S-transferase; WT, wild type.
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**RESULTS**

**Structural Characteristics of Rabip4—** Rabip4 is an endosomal protein containing the sequence C2X3C2X3(217)(221) C2X3, which is characteristic of the FYVE finger motif (shown in red in Fig. 1). This double zinc finger domain of Rabip4 is 18–52% identical to the FYVE finger of the mammalian proteins EEA1, Hrs, Akhzn, SAR, Fgd1, PIKFyve, and Rabenosyn-5. The highest homology was found with EEA1 and the lowest one with Rabenosyn-5. The cysteine residues of the core motif involved in zinc binding are conserved. The essential nucleotides involved in the binding of EEA1 with PtdIns(3)P (PtdIns(3,4)P2, PtdIns(3,5)P2) HHCRCX3CX2(3)(2)HHCRCX3CX2(3)X2(27) (Fig. 1) were identical, the percentage of identity between Rabip4 and caveolin was 36%. Since Rabip4 possesses a putative FYVE finger motif, we next determined whether it was able to bind PtdIns(3)P or PtdIns(3,4)P2 or ORF37 is not important but the blocks are enriched in hydrophobic amino acids in conserved positions (20). The secondary structure of the RUN domain was predicted to be essentially α-helical. Furthermore, the conserved basic residues in this predominantly all-α-fold might reveal a conserved tridimensional conformation that could be important for the function of the RUN domain. The FYVE Finger Motif of Rabip4 Binds PtdIns(3)P and Was Required for the Function of Rabip4—Since Rabip4 possesses a putative FYVE finger, we next determined whether it was able to bind PtdIns(3)P. Therefore, we measured the ability of GST-Rabip4 (401–600), a fusion protein between GST and the amino acids 401–600 of Rabip4, to bind to phospholipidic vesicles containing the different phosphatidylinositols (Fig. 2A). GST-Rabip4 (401–600) bound to vesicles only when they contained PtdIns(3)P, whereas GST alone did not interact with these vesicles (data not shown). This binding was highly specific, since GST-Rabip4 (401–600) did not recognize vesicles containing the same amount of PtdIns, PtdIns(3,4,5)P3.
PtdIns(3,5)P₂. Thus, the FYVE finger motif of Rabip4 specifically interacts with PtdIns(3)P.

We then compared the behavior of a Rabip4 protein mutated on the FYVE finger (Rabip4 His₅⁵⁴–His₅⁵⁵) to the wild type Rabip4. We chose to mutate the His₅⁵⁴ and His₅⁵⁵ because the corresponding mutations in EEA1 abolished its ability to interact, in vitro, with the zinc molecules required for PtdIns(3)P binding and its endosomal localization (15, 28). We compared confocal images obtained in CHO cells that expressed GFP-Rabip4 and GFP-Rabip4 His₅⁵⁴–His₅⁵⁵ to a similar level (Fig. 2B, top panels). Both GFP fusion proteins labeled punctated structures. However, the size of these vesicles was smaller when the FYVE finger was not functional (Rabip4 His₅⁵⁴–His₅⁵⁵) than with Rabip4 WT. Rabip4 overexpression was shown previously to induce the appearance of enlarged vesicles (1), and the mutation within the FYVE finger altered the ability of Rabip4 to induce this phenomenon. To strengthen further the hypothesis that the FYVE finger was involved in the function of Rabip4, we analyzed the ability of Rabip4 His₅⁵⁴–His₅⁵⁵ to induce an enlargement of endosomal structures when coexpressed with active Rab4. Although the coexpression of Rabip4 with myc-Rab4 Q67L (a constitutively active form of Rab4) led to the expansion of endosomal structures in CHO cells (Fig. 2B, right panels), such a similar enlargement was not induced by the expression of GFP-Rabip4 His₅⁵⁴–His₅⁵⁵ with active Rab4. It should be noted, however, that Rabip4 His₅⁵⁴–His₅⁵⁵ and active Rab4 are colocalized in slightly enlarged punctated structures (Fig. 2B, left panels). Taken together, these observations suggest that the FYVE finger plays a role in the function of the protein since the mutant protein did not induce a marked enlargement of the endosome, but that overexpression of the mutated protein can partially compensate for this loss of function.

Rabip4 Was a Membrane-associated Hydrophilic Protein—To determine the cellular distribution of Rabip4, CHO cells were homogenized and the membrane-associated fraction (pellet) and the cytosol were obtained by ultracentrifugation (Fig. 3A). Equal relative amounts of homogenate and both fractions were separated by SDS-PAGE before immunodetection of Rabip4. This antibody detects an endogenous protein of 68 kDa migrating with the same apparent molecular weight as the overexpressed protein (Fig. 3A, top panel). Despite its hydrophilic character, endogenous or overexpressed Rabip4, similar to Rab4, was mainly associated with the membrane fraction,
agents. By contrast, a Na$_2$CO$_3$, pH 10.5, treatment detached treatment were highly resistant to high ionic strength and chaotropic interactions between Rabip4 or Rab4 and the membrane frac-
ylthioglucoside), whereas Rab4 was efficiently solubilized. The

bar cells, and the membrane-anchored protein Rab4 was still present in the

membrane fraction. These observations indicated that Rabip4 is a peripheral protein tightly associated with membranes as well as with Triton X-100-insoluble structures.

To determine the nature of the association between Rabip4 and membranes, the pellet fractions obtained from control or Rabip4 overexpressing cells were submitted to various treat-
ments, i.e. 1% Triton X-100, 60 mM β-octylthioglucoside, 1 M NaCl, 4 M urea, or 10 mM Na$_2$CO$_3$, pH 10.5 (Fig. 3A). Rabip4 was partly resistant to the detergents (Triton X-100 and β-octylthioglucoside), whereas Rab4 was efficiently solubilized. The interactions between Rabip4 or Rab4 and the membrane fraction were highly resistant to high ionic strength and chaotropic agents. By contrast, a Na$_2$CO$_3$, pH 10.5, treatment detached Rabip4 from the pellet fraction, whereas the geranylgeranyl membrane-anchored protein Rab4 was still present in the membrane fraction.
of Rabip4 was involved in the subcellular localization of Rabip4.

To confirm that the FYVE finger was not sufficient for the targeting of Rabip4 to the membrane-associated fraction, CHO cells expressing myc-Rabip4 were treated or not with wortmannin, an inhibitor of PtdIns(3) kinase. Cells were then fractionated into cytosol and membrane pellets or analyzed by confocal microscopy. Both analyses indicated that wortmannin did not modify the association of Rabip4 with the membrane fraction, whereas it induced the redistribution of EEA1 from the cytosol (Fig. 4A). We reported previously (Fig. 3) that the myc-Rabip4 protein associated with the membrane fraction was only partly detergent-soluble. We studied whether a wortmannin treatment modified this property. The membrane-associated fraction obtained from control or wortmannin-treated cells was treated with 1% Triton X-100 before recovering the soluble and insoluble fractions by ultracentrifugation, and the pellet was treated with 1% Triton X-100. Solubilized and non-solubilized material was then analyzed by Western blotting using anti-Myc and anti-EEA1 antibodies. Representative autoradiograms are shown (left). C, wortmannin modifies GFP-Rabip4 labeling. CHO cells stably expressing GFP-Rabip4 were treated without (control) or with 100 nm wortmannin for 30 min. Cells were analyzed by confocal fluorescent microscopy as described under “Experimental Procedures.” The bar represents 1 μm.

**FIG. 5. The N terminal of Rabip4 is involved in the Triton X-100 insolubility of the protein.** A, the membrane-associated myc-Rabip4 (1–212) is detergent-insoluble. CHO cells were transiently transfected with pcDNA3-myc-Rabip4 (1–212). Cells were homogenized and submitted to subcellular fractionation and Triton X-100 solubilization as described in Figs. 3 and 4. The presence of myc-Rabip4 (1–212) was detected in each fraction by using anti-Myc antibodies. B, Myc-Rabip4 (1–212) is present in GFP-Rabip4-containing vesicles. CHO cells were cotransfected with pcDNA3-myc-Rabip4 (1–212) and eGFP-Rabip4. Cells were treated for confocal analysis as described under “Experimental Procedures.” myc-Rabip4 (1–212) was detected using an anti-Myc antibody followed by Texas Red-coupled anti-mouse antibodies. The figure shows the merged image of GFP-Rabip4 (in green) and myc-Rabip4 (1–212) (in red); the yellow color results from the overlay of green and red. The bar represents 1 μm. In the inset an enlargement of a cellular part shows the punctated structures aligned on a network with a honeycomb appearance.

### The N-terminal Part of Rabip4, Containing a RUN Domain, Is Involved in Its Association with the Triton X-100-insoluble Fraction

To determine which domain of Rabip4 was required for its association with the Triton X-100-insoluble material, we examined whether Rabip4 (1–212), which appears to trigger Rabip4 association to the membranes, was also responsible for the partial Triton X-100 insolubility. CHO cells expressing myc-Rabip4 (1–212) were submitted to subcellular fractionation, and the pellet was treated with 1% Triton X-100. Solubilized and non-solubilized material was then analyzed by Western blot using an anti-Myc antibody. The totality of the membrane-associated myc-Rabip4 (1–212) was recovered in the Triton X-100-insoluble fraction (Fig. 5A). To strengthen the fact that the amino acids 1–212 of Rabip4 were involved in the targeting of Rabip4 to its intracellular localization, we looked for the colocalization of Rabip4 and Rabip4 (1–212) when over-expressed in CHO cells (Fig. 5B). We observed that GFP-Rabip4 was colocalized with myc-Rabip4 (1–212) in punctated structures as indicated by their yellow color, with very few green structures containing only GFP-Rabip4. Further myc-Rabip4 (1–212) labeling defined a filamentous network giving rise to a honeycomb appearance as emphasized in the enlarged square of Fig. 5B and already visible in Fig. 4C in cells treated with wortmannin. Taken together, these results suggest that the N-terminal part of Rabip4 first determines the subcellular localization of the protein, whereas other domains, such as the FYVE finger, might be secondary to other types of interactions around its previous localization.

In accordance with the fact that the FYVE finger of Rabip4
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was not the only determinant for its endosomal localization, we noticed that EEA1 and GFP-Rabip4 define different microdomains at the level of the same endosomal vesicles (Fig. 6). Although GFP-Rabip4 was present together with EEA1 in the same endosomes (nearly all the vesicles were positive for both GFP-Rabip4 and EEA1), we observed at a higher magnification that EEA1 (red) and GFP-Rabip4 (green) labeled different regions within endosomes. By contrast, GFP-Rabip4 and Rab4 uniformly labeled the endosomes that were enlarged by the overexpression of wild type Rab4. Similar types of observations have already led to the conclusion that in the same endosomal vesicle Rab5 (whose effector is EEA1) and Rab4 were present in distinct microdomains (29).

Rabip4 Was Not Present in Lipid Rafts—Lipid rafts are microdomains found at the plasma membrane and intracellular membrane organelles, which are experimentally recovered in a detergent-insoluble fraction (30). To test whether Rabip4 was present in these microdomains, we first searched for a colocalization of Rabip4 with proteins known to be enriched in lipid rafts (Fig. 7). By immunofluorescence confocal analysis, we found that GFP-Rabip4 (green) did not colocalize with endogenous caveolin (red) and that myc-Rabip4 (red) did not colocalize either with a GFP-GPI-anchored protein (green). Furthermore, when lipid rafts were purified by a flotation assay, endogenous caveolin floated on the sucrose cushion while Rabip4 remained at the bottom of the tube (Fig. 7B). Rabip4 was not colocalized with caveolin or a GPI-anchored protein. CHO cells were transiently transfected with pEGFP-Rabip4 (left) or cotransfected with pEGFP-GPI and pcDNA3-myc-Rabip4. Cells were treated for confocal analysis as indicated under “Experimental Procedures.” Endogenous caveolin was detected using anti-caveolin monoclonal antibodies, and myc-Rabip4 was detected with anti-Myc monoclonal antibodies followed by Texas Red-coupled anti-mouse antibodies. The total lysate (1/3 of the total material) was analyzed in parallel. A typical autoradiogram representative of 3 independent experiments is shown.

DISCUSSION

We have characterized the implication of various domains of Rabip4 for the protein subcellular localization and function. A series of evidences suggest that the FYVE finger of Rabip4 and the Rab4-binding region are not sufficient to trigger the protein to its endosomal localization, although they play a role in its ability to enlarge the size of early endosomes. First, the protein was associated to endosomes, even when null mutations were
performed into the FYVE finger, as evidenced by confocal microscopy or following fractionation (data not shown). However, these mutations are not silent since the inactivation of the FYVE finger decreases the ability of Rabip4 to enlarge endosomes when expressed together with active Rab4. Second, the C terminus of Rabip4, Rabip4-(401–600), which contains the FYVE finger and the Rab4-binding domain, is cytosolic. Third, a wortmannin treatment that decreases the intracellular level of PtdIns(3)P does not increase the amount of Rabip4 in the cytosol, whereas a similar treatment inhibits the endosomal localization of EEA1 (16, 32) and Rabenosyn-5 (33). These observations indicate that the FYVE finger of Rabip4 was not the major determinant for its endosomal localization. Thus we observed that Rabip4 and EEA1 are not totally colocalized within a single endosomal vesicle. This highly suggests that another domain(s) of Rabip4 was responsible for its association with endosomal microdomains that do not necessarily contain PtdIns(3)P. It should be noted that this endosomal localization was not due either to the protein region implicated in the Rab4 interaction since, as noted above, Rabip4-(401–600) contains both the FYVE finger and amino acid sequences needed for Rab4 binding (1) and is cytosolic.

Our results point to an important role of the N-terminal domain of Rabip4 in the subcellular localization of the protein. We show that the N-terminal part of Rabip4 (amino acids 1–212) not only associates with endosomes containing Rabip4 but also with a filamentous network of an unidentifiable nature. This part of the molecule contains the newly described RUN domain, which was predicted to constitute the “core” of a globular structure that possibly interacts with other partners. Since it was found in RIPP8, a protein that interacts with the small GTPase Rap2 (19), in a Rab6 effector (ORF37) (27) and in proteins containing FYVE finger (including Rabip4), it has been proposed that the RUN domain might play a role in relation to small GTPases signaling (20). It was suggested that it could be of use to bind small GTPases, since the RUN domain of RPIP8 was interacting with Rap2. It is perhaps not the function of a RUN domain to bind any form of Rab4 (1), Rab5, and Rab11,2 the three Rabs enriched in early endosomes, nor Rap2 (1). In view of our results, we suggest that the RUN domain might be responsible for an interaction with a filamentous network of an unidentified nature but is probably linked to the actin cytoskeleton, since the disorganization of actin by cytoskeleton was profoundly modified the labeling obtained with Rabip4. The proteins that form these filaments are not known yet but would also be present at the surface of endosomes since Rabip4-(1–212) was colocalized with Rabip4 WT on endosomes. This colocalization was not due to a dimerization of the two proteins. Indeed, while Rabip4 homodimerized in the yeast two-hybrid system, Rabip4 WT and (1–212) did not.2

As discussed above, the FYVE finger and the Rab4-binding site are not sufficient for the association of Rabip4 with endosomes, although those domains play a role in the partitioning of the proteins between the detergent-soluble and -insoluble fractions. In the presence of wortmannin, most of the protein is Triton X-100-insoluble. Furthermore, both Rabip4 mutated on the FYVE finger and Rapip4Δ(507–517), a form of Rapip4 unable to interact with active Rab4 (1), are found only in the Triton X-100-insoluble fraction.3 The amounts of Rabip4 present in the membrane detergent extracts are tightly dependent on the presence of PtdIns(3)P and probably of Rab4. Both types of interaction are required since the destruction of only one of these interactions renders Rabip4 totally detergent-insoluble. This would indicate that either the binding of Rabip4 to

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2 M. Mari, Y. Le Marchand-Brustel, and M. Cormont, unpublished observations.
PtdIns(3)P through its FYVE finger is required for a subsequent interaction with Rab4, as described for Rab5 and EEA1 (16), or that the binding of Rabip4 with active Rab4 stabilizes the interaction between the FYVE domain and PtdIns(3)P.

We suggest that Rabip4 is triggered in endosomal microdomains enriched in some unidentified cytoskeletal elements. This localization would allow for an efficient recruitment of Rabip4 to the PtdIns(3)P-enriched area of the endosomes (14) and for a concomitant recruitment of active Rab4 to permit the enlargement of endosomes. We don’t know yet whether the filamentous structures are necessary for some intracellular movements of endosomes or if they are only needed for a correct targeting of Rabip4. More studies will be necessary to get a better knowledge of the potential partners and/or roles of the RUN domains.

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REFERENCES

1. Cormont, M., Mari, M., Galmiche, A., Hofman, P., and Le Marchand-Brustel, Y. (2001) Proc. Natl. Acad. Sci. U. S. A. 98, 1637–1642
2. Mills, I., Jones, A., and Clague, M. (1998) Curr. Biol. 8, 881–884
3. Simonsen, A., Gaullier, J.-M., d’Arrigo, A., and Stenmark, H. (1999) J. Biol. Chem. 274, 28857–28866
4. Lupas, A. (1996) Trends Biochem. Sci. 21, 375–382
5. Gillooly, D. J., Simonsen, A., and Stenmark, H. (2001) Biochem. J. 355, 249–258
6. Burd, C. G., and Emr, S. D. (1998) Mol. Cell 2, 157–162
7. Stenmark, H., and Aasland, R. (1999) J. Cell Sci. 112, 4175–4183
8. Mu, F. T., Callaghan, J. M., Steele-Mortimer, O., Stenmark, H., Parton, R. G., Campbell, P. L., McCluskey, J., Yes, J. P., Toek, E. F., and Toh, B. H. (1995) J. Biol. Chem. 270, 13503–13511
9. Yamamoto, A., DeWald, D. B., Boronenkov, I. V., Anderson, R. A., Emr, S. D., and Koshland, D. (1996) Mol. Biol. Cell 7, 525–539
10. Piper, R. C., Cooper, A. A., Yang, H., and Stevens, T. H. (1995) J. Biol. Cell. 131, 603–617
11. Webb, G. C., Zhang, J., Garlow, S. J., Wesp, A., Riezman, H., and Jones, E. W. (1997) Mol. Biol. Cell 8, 871–895
12. Gaullier, J., Simonsen, A., d’Arrigo, A., Brennes, B., and Stenmark, H. (1998) Nature 394, 432–433
13. Patki, V., Lawe, D. C., Corvera, S., Virbasius, J. V., and Chawla, A. (1998) Nature 394, 433–434
14. Gillooly, D. J., Morrow, I. C., Lindsay, M., Gould, R., Bryant, N. J., Gaullier, J.-M., Parton, R. G., and Stenmark, H. (2000) EMBO J. 19, 4577–4588
15. Stenmark, H., Aasland, R., Toh, B.-H., and d’Arrigo, A. (1996) J. Biol. Chem. 271, 24048–24054
16. Lawe, D. C., Patki, V., Heller-Harrison, R., Lambright, D., and Corvera, S. (2000) J. Biol. Chem. 275, 3699–3705
17. Komada, M., Masaki, R., Yamamoto, A., and Kitamura, N. (1997) J. Biol. Chem. 272, 20538–20544
18. Miura, S., Takeshita, T., Asao, H., Kimura, Y., Murata, K., Sasaki, Y., Hanai, J. I., Beppu, H., Tsukazaki, T., Watanabe, J. L., Miyazono, K., and Sugamura, K. (2000) Mol. Cell. Biol. 20, 9346–9355
19. Janoueix-Lerosey, I., Pasheva, E., de Tand, M., Tavitian, A., and de Gunzburg, J. (1998) EMBO J. Biol. Chem. 272, 2980–2988
20. Callebaut, I., le Gunsburg, J., Goud, B., and Monon, J.-P. (2001) Trends Biochem. Sci 26, 79–83
21. Bertoluzza, M. N., Cormont, M., Gautier, N., Van Obberghen, E., and Le Marchand-Brustel, Y. (1996) Diabetologia 39, 889–906
22. Hope, M. J., Bally, M. B., Webb, G., and Cullis, P. R. (1985) Biochim. Biophys. Acta 812, 55–65
23. Paris, S., Bercaud-Dufour, S., Robineau, S., Bigay, J., Antonny, B., Chahre, M., and Chardin, P. (1997) J. Biol. Chem. 272, 22221–22226
24. Vidalain, P. O., Azocar, O., Seret-Delprat, C., Kabourdin-Combe, C., Gerlier, D., and Manie, S. (2000) EMBO J. 19, 3304–3313
25. Kutateladze, T. G., Ogura, K. D., Watson, W. T., de Beer, T., Emr, S. D., Burd, C. G., and Overduin, M. (1999) Mol. Cell 3, 805–811
26. Kutateladze, T., and Overduin, M. (2001) EMBO J. 19, 28857–28860
27. Janoueix-Lerosey, I., Jollivet, F., Camonis, J., Marche, P., and Goud, B. (1995) J. Biol. Chem. 270, 14901–14908
28. Gaullier, J. M., Ronning, E., Gillooly, D. J., and Stenmark, H. (2000) J. Biol. Chem. 275, 24595–24600
29. Sonnichsen, B., De Renzis, S., Nielsen, E., Riedorf, J., and Zerial, M. (2000) J. Cell Biol. 149, 901–914
30. Jacobson, K., and Dietrich, C. (1999) Trends Cell Biol. 9, 87–91
31. Spector, I., Braet, F., Shochet, N. R., and Bulb, M. (1999) Microsc. Res. Tech. 47, 18–37
32. Simonsen, A., Lippe, R., Christoforidis, S., Gaullier, J.-M., Brech, A., Callaghan, J., Toh, B. H., Murphy, C., Zerial, M., and Stenmark, H. (1998) Nature 394, 494–498
33. Nielsen, E., Christoforidis, S., Utenweiler-Joseph, S., Miczynska, M., Dewitte, F., Wilm, M., Hoflacque, B., and Zerial, M. (2000) J. Cell Biol. 151, 601–612
